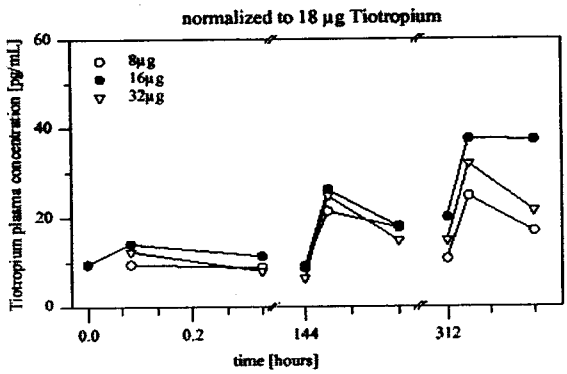
Table 1. *Geometric mean PK parameters after inhalation solution via BINEB device*

		dose	8.0 µg		16 µg		32 µg	
		n	gMean	% gCV	gMean	% gCV	gMean	% gCV
Day 1								
C_{5min}	[pg/mL]	6/5/8	4.23	37.8	12.5	28.9	22.1	60.2
NC_{5min}	[pg/mL] for 18 µg	6/5/8	9.52	37.8	14.1	28.9	12.5	60.2
C_{24h}	[pg/mL]	3/5/7	4.26*	18.8	5.01	61.8	7.10*	46.8
NC_{24h}	[pg/mL]	3/5/9	9.59	18.8	5.64	61.8	3.99	46.8
$AUC_{0-20\,min}$	[pg.h/mL]	-/-/7	--	--	--	--	4.78	51.4
$NAUC_{0-20\,min}$	[pg.h/mL] for 18 µg	-/-/7	--	--	--	--	2.69	51.4
Ae_{0-4h}	[% of dose]	9/9/9	1.49	75.7	2.99	104	2.24	91.4
Ae_{0-24h}	[% of dose]	8/9/9	5.66	36.1	8.41	61.1	6.45	55.2
Day 7								
C_{5min}	[pg/mL]	9/7/8	9.51	46.8	23.2	23.7	44.2	63.1
NC_{5min}	[pg/mL] for 18 µg	9/7/8	21.4	46.8	26.1	23.7	24.8	63.1
C_{pre}	[pg/mL]	4/7/9	4.05	77.7	7.81	27.5	11.7	50.6
NC_{pre}	[pg/mL]	4/7/9	9.11	77.7	8.79	27.6	6.56	50.6
$AUC_{0-20\,min}$	[pg.h/mL]	4/5/7	3.74	62.3	6.10	22.0	10.4	59.4
$NAUC_{0-20}$	[pg.h/mL] for 18 µg	4/5/7	8.42	62.2	6.86	21.9	5.87	59.4
Ae_{0-4h}	[% of dose]	9/9/9	4.54	43.6	7.62	59.5	6.82	64.0
Ae_{0-24h}	[% of dose]	-/7/4	--	--	29.1	27.6	29.4	28.7
Day 14								
C_{5min}	[pg/mL]	8/6/7	11.0	68.0	33.3	33.1	56.5	50.3
NC_{5min}	[pg/mL] for 18 µg	8/6/7	24.7	68.0	37.4	33.1	31.8	50.3
C_{pre}	[pg/mL]	7/7/7	4.75	69.0	17.9	34.5	26.7	56.4
NC_{pre}	[pg/mL] for 18 µg	7/7/7	10.7	69.0	20.1	34.5	15.0	56.4
$AUC_{0-20\,min}$	[pg.h/mL]	5/4/5	3.64	63.3	10.6	17.8	14.4	53.5
$NAUC_{0-20min}$	[pg.h/mL] for 18 µg	5/4/5	8.18	63.3	11.9	17.8	8.12	53.5
Ae_{0-4h}	[% of dose]	9/9/8	5.41	38.1	7.69	47.6	7.40	56.7
Ae_{0-24h}	[% of dose]	9/9/7	20.1	25.5	24.5	34.0	21.3	53.3

Source data in U97-2426, *values of BLQ replaced by ½ BLQ

Figure 1. Geometric mean tiotropium plasma concentrations after inhalation of tiotropium solution via BINEB device by different groups of healthy male subjects.



Conclusions: The relative bioavailability of tiotropium via the [redacted] device was higher than with dry powder inhalation (27-33% *vs* 19.5%). The amount excreted unchanged on Day 14 was 20.1-24.5% in these healthy male subjects.

Protocol 205.114/117 (Study Report #U99-3169)

Study Type: Multiple dose PK and PD in COPD patients (Phase III)
Title: A multiple dose comparison of 18 µg of tiotropium inhalation capsules and placebo in a one year, double-blind, safety and efficacy study in adults with chronic obstructive pulmonary disease (COPD). Initial 13 weeks of study.
Clinical Investigators: [redacted]
Objectives: To evaluate the bronchodilator properties of tiotropium in patients with chronic obstructive pulmonary disease. Some plasma and urine samples were collected to evaluate the preliminary PK of tiotropium in target population.
Study design: This was a one-year, multi-center, randomized, double-blind, parallel groups study to compare the long-term bronchodilator efficacy and safety of tiotropium 18 µg inhalation capsules vs placebo in patients with COPD (total study population consisted of 470 patients). Following an initial screening, patients entered a two-week baseline period. Patients who successfully completed this phase were randomized into the ore-year, double-blind portion of the study in which they received either tiotropium or placebo once daily in the morning. For patients taking theophylline drug levels were measured prior to pulmonary function testing.

Pulmonary function tests and adverse events were recorded accoring to the protocol. Plasma and urine were collected at ten centers. Tiotropium concentrations and excretion data were grouped and compared according to age, gender, renal function and lung function.
Subjects: Urinary excretion data and/or tiotropium plasma concentrations were available from 118 patients (75 male and 43 female) with a mean age of 63.8 years, a mean weight of 77.4 kg, a mean FEV1 of 1.17 L (5 mm predose) and a mean predicted creatinine clearance of 78.5 mL/min.
Formulations:

- 18.0 µg tiotropium (batches PD-1732 and PD-1742 = 9603001, 22% [redacted] or
- placebo inhalation capsules (batches PD-1734 and PD-1743 = 9602001)

The inhalation capsules were administered in the morning by Handihaler device. Breath was held as long as it was comfortable after inhalation. Inhalation was done twice from each capsule in order to empty the capsule completely. The sponsor indicated that the dose of 18 µg was selected based on the results of tiotropium dose ranging study 205.108 in COPD patients.
Sampling times:
PK: Tiotropium plasma concentrations were determined 5 minutes predose, 5 minutes and 2 hours post dose at visits 5 (day 50) and 7 (day 92). Tiotropium excretion in urine was measured at visits 4 (day 29) and 6 (day 71) in fractions –2 – 0 hour before and 0 – 2hours after inhalation. Complete 24-hour urine fractions were collected at visits 5, 7 and 9 (day 175).
PD: Pulmonary function testing (FEV_1 and FVC) was conducted 1 hr prior to and just prior to dosing and at 30, 60, 180 min after drug administration at 1, 7, 13, 25, 37 and 49 weeks of therapy. Primary endpoint was measurements of trough FEV_1 response at 13 weeks after initiation of treatment. Trough FEV_1 was defined as the mean of the two FEV1 readings at the end of the dosing interval, at approximately 23 to 24 hrs post drug administration. Clinically meaningful difference between tiotropium and placebo was defined as 56 mL or more of FEV_1.
Analytical Methodology:
Assay Method: Plasma and urine samples were assayed by a revalidated [redacted] assay with a limit of quantification of [redacted] pg/mL for plasma and [redacted] pg/mL for urine samples.
Accuracy and Precision: Assay precision in plasma and urine samples during sample analysis was within [redacted] and [redacted] for precision and [redacted] and ± [redacted] for accuracy, respectively.

Results:

Plasma: The sponsor stated that tiotropium predose concentrations were expected to be at the limit of quantification or below. Therefore, 56% and 47% of pre-dose samples on Days 50 and 92 respectively were analyzed. Also stated that concentrations below the quantification limit were replaced by ½ the LQ. The geometric mean data are summarized in Table 1.

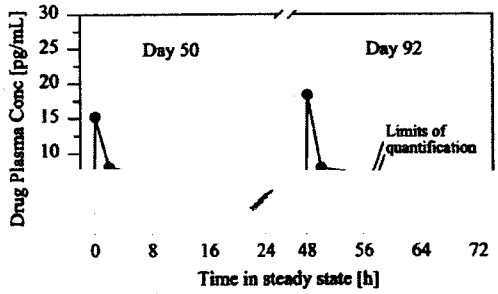
Table 1. Geometric mean plasma concentrations and urinary excretion following once daily 18 µg doses by DPI

Day Visit		50 5	92 7	175 9	29 4	71 6
C_{-5min}	[pg/mL]	5.61 (3.16#)	6.36 (3.28#)	--	--	--
	(%gCV, n)	(71, 24, 22$)	(78, 30, 21$)	--	--	--
C_{+5min}	[pg/mL]	17.3	19.1	--	--	--
	(%gCV, n)	(65, 84)	(62, 86)	--	--	--
C_{2h}	pg/mL	8.72	8.12	--	--	--
	(%gCV, n)	(48, 96)	(48, 103)	--	--	--
Ae_{0-24h}	% of dose	7.08	6.88	7.20	--	--
	(%gCV, n)	(48, 99)	(53, 88)	(51, 101)	--	--
Ae_{-2-0h}	% of dose	--	--	--	0.390	0.398
	(%gCV, n)	--	--	--	(61, 69)	(72, 70)
Ae_{0-2h}	% of dose	--	--	--	1.29	1.23
	(%gCV, n)	--	--	--	(69, 71)	(81, 68)

Source data: TABLEs 13: 2 and 13: 3, values below limit of quantification omitted
#:These geometric mean values were obtained, when values below the limit of quantification were replaced by ½ the limit of quantification. $: the number of replaced values in this case.

It can be concluded that drug plasma concentrations were about 4 pg/mL (3 - 6 pg/mL) at predose conditions in steady state, rose to about 17 pg/mL at 5 minutes (15 - 19 pg/mL) after inhalation and declined to about 8 pg/mL two hours after inhalation. This can be interpreted that steady state concentrations swing by a factor of 4.5 during the day in COPD patients. Concentrations decline to about twice the predose value within 2 hours after administration and fall then slowly over 22 hours again to the level of the predose concentrations. The concentration-time profile (expected) in plasma is shown in Figure 1. The profile is characterized by a short peak and relatively constant concentrations over 22 h (92 % of the day). However, the maximum concentrations may occur earlier than 5 min after inhalation.

Figure 1. Geometric mean tiopropium plasma concentration-time profile (expected)



Urine: Urine was collected fractions, 0-2 h (represent absorption phase), -2-0 h (elimination phase) and 0-24 h at steady state. Table 1 shows that there is no relevant change in urinary excretion from day 29 to day 71 and not from day 50 to day 92 or 175. The elimination half-life of tiotropium is in the range of 5 - 6 days and day 29 should therefore represent a steady state sample, which was confirmed. Thus steady state was stable over at least 175 - 29 = 146 days or about 5 months. The 2 hr postdose urine samples showed 3.1 and 3.3 more tiotropium excretion

in comparison to the 2 hr predose samples. Urine fraction 0-24 hr, 0-2 hr and -2 – 0 h of a steady state interval were about 7%, 1.2- 1.3 and 0.4% of the dose, respectively.

Effect of Gender on tiotropium PK: Plasma and urine data are tabulated per gender in Table 2 and 3, respectively.

Table 2. Effect of Gender on Geometric Mean (% gCV) Tiotropium Plasma Concentrations Following once Daily Inhalation of 18 µg Tiotropium by COPD Patients

	Visit	5			7		
	Day	50			92		
	Time	-5 min*	5 min	2 h	-5 min*	5 min	2 h
Male	N	17 / 29	54	59	16 / 32	52	64
Patients	gMean	6.05 / 3.61	16.7	7.65	6.92 / 2.98	17.6	7.41
(64 y, 44 - 85 y)	% gCV	74.3 / 100	64.2	43.1	86.6 / 133	64.2	42.8
Female	N	7 / 17	30	37	14 / 19	34	39
Patients	gMean	4.65 / 2.50	18.6	10.7	5.77 / 3.84	21.4	9.45
(63 y, 40 - 83 y)	% gCV	63.9 / 79.0	68.2	47.3	70.3 / 109	56.3	51.1
ratio#		0.769/0.693	1.11	1.40	0.834/1.29	1.22	1.28

BLQ values not replaced, Source data in TABLE 13: 2.3
#: male – reference
*: the right figures represents the figures with BLQ values replaced by ½ the limit of quantification

Table 3. Effect of Gender on Geometric Mean (% gCV) Tiotropium excretion in urine (% of dose) following once Daily Inhalation of 18 µg Tiotropium by COPD Patients

Visit	4	4	6	6	5	7	9
Day	29	29	71	71	50	92	175
Fraction	-2 - 0 h	0 - 2 h	-2 - 0 h	0 - 2 h	0 - 24 h	0 - 24 h	0 - 24 h
Male, N	46	48	50	47	60	57	61
gMean	0.393	1.38	0.404	1.12	7.12	7.29	7.57
% gCV	61.7	65.8	78.6	90.5	38.2	38.5	42.1
Female, N	23	23	20	21	39	31	40
gMean	0.385	1.105	0.383	1.54	7.01	6.19	6.67
% gCV	61.9	74.8	54.3	50.4	62.6	74.6	62.7
ratio#	0.980	0.801	0.948	1.38	0.985	0.849	0.881

Source data: TABLE 13: 3.3
#: male – reference

In Table 2, there was a slight to moderate trend to higher post dose drug plasma concentrations in female patients vs male patients, while predose concentrations gave no consistent ratios for days 50 and 92. The sponsor speculates that the latter may be due to the issue of values below the limit of quantification. The sponsor indicated that a trend to higher drug plasma concentrations in female patients could be due to the lower body weight of female patients and differences in the body composition (fat/muscle etc). Overall demographic database for the patients in the pharmacokinetie evaluation showed a median weight of 65.3kg for female patients (N 43), while male patients (N = 75) showed a median weight of 78.2 kg. There were no relevant differences in urinary excretion between male and female patients.

Effect of Age on tiotropium PK: Drug plasma concentrations seemed to be relatively constant for the age groups 50 - 59 and 60 - 69 years, while the oldest age group (>69 years) showed a trend to about 30 -40 % higher drug concentrations 2 hrs after inhalation (Table 4), and the sponsor indicated that this could be due to decrease in renal function with old age (note that decrease in CLcr). There was no apparent trend in maximum drug concentrations 5 minutes after inhalation. The effect of age on the urinary excretion of tiotropium is shown in Table 5. The sponsor selected the age group 50 - 59 years for reference purposes because the small number of observations in the age group 40 - 49 years.

Table 4. *Effect of Age on Geometric mean Tiotropium Plasma Concentrations (pg/mL) and* mean creatinine clearance (CLcr)

age range [years]	N	CL_{CR} Mean [mL/min]	Day 50 C_{-5min}	Day 50 C_{5min}	Day 50 C_{2h}	Day 92 C_{-5min}	Day 92 C_{5min}	Day 92 C_{2h}
40 – 49	9	101	(15.2)	15.3	7.91	(2.64)	18.8	8.45
		%gCV, N:	169, 2	63.3, 7	30.4, 9	– 1	69.7, 6	48.4, 7
50 – 59	26	92.1	6.73	19.2	8.22	10.7	20.5	7.23
		%gCV, N:	75.0, 4	72.7, 20	52.1, 24	126, 4	55.0, 20	43.4, 24
60 – 69	52	80.0	4.65	16.1	7.77	6.54	16.4	7.78
		%gCV, N:	59.7, 10	72.3, 38	41.7, 39	86.1, 14	58.9, 38	46.7, 42
>69	31	58.0	5.03	19.0	11.6	3.51	23.2	9.37
		%gCV, N:	47.5, 8	44.5, 19	48.4, 24	43.3, 11	66.1, 22	50.1, 30

BLQ values not replaced. Source data in TABLE 13: 2.5

Table 5. Effect of Age on mean (% gCV) tiotropium excretion in urine (% of dose)

Visit	4	4	6	6	5	7	9
Day	29	29	71	71	50	92	175
Fraction	-2 - 0 h	0 - 2 h	-2 - 0 h	0 - 2 h	0 - 24 h	0 - 24 h	0 - 24 h
40 - 49 years							
gMean	0.597	2.02	0.332	1.45	6.63	5.90	6.89
(% gCV, N)	(33.7, 6)	(51.6, 5)	(204, 6)	(117, 5)	(66.2, 6)	(41.4, 3)	(62.7, 6)
50 - 59 years							
gMean	0.379	1.46	0.365	1.66	7.01	5.92	7.26
(% gCV, N)	(59.7, 16)	(82.2, 16)	(43.6, 15)	(59.0, 16)	(38.3, 23)	(45.9, 20)	(48.6, 25)
60 - 69 years							
gMean	0.354	1.32	0.397	1.22	6.95	6.79	7.65
% gCV, N	(60.8, 30)	(54.2, 31)	(63.9, 33)	(69.6, 30)	(45.2, 42)	(63.1, 38)	(47.2, 43)
>69 years							
gMean	0.409	0.978	0.464	0.912	7.43	7.97	6.55
% gCV, N	(67.5, 17)	(76.8, 19)	(72.0, 16)	(99.6, 17)	(58.9, 28)	(41.3, 27)	(57.8, 27)
Ratios vs 50 - 59 y							
40 - 49 years[#]	(1.58)	(1.38)	(0.910)	(0.873)	(0.946)	(0.997)	(0.949)
50 - 59 years[#]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
60 - 69 years[#]	0.934	0.904	1.09	0.735	0.991	1.15	1.05
>69 years[#]	1.08	0.670	1.27	0.549	1.06	1.35	0.902

Source data: TABLE 13: 3.5

#: The group 50 - 59 years was selected for reference because of the small number of observations in the group 40 - 49 years.

Overall, urinary excretion seemed to be delayed in the elderly but was overall constant (e.g., there was no change in total urinary excretion of tiotropium as seen from the geometric mean ratio for the 24 hr collections on days 50, 92 and 175).

Effect of Renal function on tiotropium PK: The effect of renal function was based on measured CLcr.

Table 6. Effect of measured creatinine clearance on Geometric mean Tiotropium Plasma Concentrations (pg/mL)

Creatinine clearance group	N	CL_{CR} Mean [mL/min]	Day 50 C_{-5min}	Day 50 C_{5min}	Day 50 C_{2h}	Day 92 C_{-5min}	Day 92 C_{5min}	Day 92 C_{2h}
30 - 50	8	41.2	2.21	17.0	16.1	3.59	37.1	10.4
mL/min		%gCV, n	(65.8, 5)	(126, 7)	(50.9, 7)	(129, 5)	(24.0, 4)	(56.3, 7)
50 - 80	52	66.4	2.97	22.3	8.34	3.12	23.7	8.75
mL/min		%gCV, n	(85.2, 20)	(73.4, 35)	(62.7, 47)	(114, 29)	(50.3, 40)	(45.4, 45)
>80	55	110	3.64	10.6	5.68	2.85	12.9	6.50
mL/min		%gCV, n	(109, 21)	(101, 45)	(65.3, 54)	(120, 15)	(78.0, 41)	(64.2, 52)
ratios vs	>80	mL/min						
30 - 50		mL/min	0.607	1.60	2.83	1.27	2.88	1.60
50 - 80		mL/min	0.816	2.10	1.47	1.10	1.84	1.35
>80		mL/min	1.00	1.00	1.00	1.00	1.00	1.00

BLQ values replaced by ½ BLQ, Source data in TABLE 13: 2.6

Table 7. *Effect of measured creatinine clearance on mean (% gCV) tiotropium excretion in urine* (% of dose) following once Daily Inhalation of 18 µg Tiotropium by COPD Patients.

Visit	4	4	6	6	5	7	9
Day	29	29	71	71	50	92	175
Fraction	-2 - 0 h	0 - 2 h	-2 - 0 h	0 - 2 h	0 - 24 h	0 - 24 h	0 - 24 h
30 - 50 mL/min							
gMean	0.240	1.42	0.564	2.03	6.47	4.79	4.23
% gCV, N	(38.5, 4)	75.0, 6	(78.5, 6)	(53.1, 6)	(49.6, 6)	(70.4, 7)	(73.7, 7)
50 - 80 mL/min							
gMean	0.375	1.22	0.384	1.20	6.41	7.12	6.81
% gCV, N	(60.6, 31)	(68.1, 31)	(63.5, 29)	(88.8, 26)	(46.4, 44)	(38.5, 38)	(45.2, 45)
>80 mL/min							
gMean	0.423	1.32	0.380	1.15	8.20	7.14	8.52
% gCV, N	(63.4, 32)	(74.0, 32)	(78.6, 33)	(78.7, 34)	(41.6, 47)	(60.8, 42)	(43.8, 46)
Ratios vs							
>80 mL/min							
30 - 50 mL/min	0.567	1.08	1.48	1.77	0.789	0.671	0.496
50 - 80 mL/min	0.887	0.924	1.01	1.04	0.782	0.997	0.799
>80 mL/min	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Source data: TABLE 13: 3.6
Data for patients 1 - 3 with a creatinine clearance <30 mL/min are given in TABLE 13: 3.6

Tiotropium is mainly renally excreted and thus it is expected that higher drug concentrations and/or lower urinary excretion. However, the data (Table 6 and 7) showed that this trend was not relevant unless CLcr < 50 mL/min.

Effect of Lung function on tiotropium PK: As shown in Table 8, there was no consistent effect of the decrease in lung function on tiotropium plasma concentrations or on the urinary excretion of tiotropium (many predose concentrations included replaced values).

Table 8. Effect of Lung function on Geometric mean tiotropium plasma concentrations (pg/mL)

FEV_1 Group	N	FEV_1 gMean [mL/min]	Day 50			Day 50
			C_{-5min}	C_{5min}	C_{2h}	Ae_{0-24h}
<0.80 L	23	0.575	2.81	14.1	7.96	7.75
		%gCV, n	(72.5, 8)	(101, 16)	(52.7, 20)	(43.8, 20)
0.80 - 1.25 L	49	0.993	2.57	15.5	8.40	6.66
		%gCV, n	(73.5, 17)	(125, 41)	(72.5, 46)	(55.2, 39)
1.25 - 1.50 L	20	1.36	3.36	16.3	5.98	6.49
		%gCV, n	(90.5, 8)	(63.1, 14)	(71.7, 20)	(44.6, 17)
>1.50 L	26	1.81	4.26	11.9	6.02	7.71
		%gCV, n	(136, 13)	(115, 19)	(72.7, 25)	(42.4, 23)
ratios vs	>1.5 L					
< 0.80	L		0.660	1.18	1.32	1.01
0.80 - 1.25	L		0.603	1.30	1.40	0.864
1.25 - 1.50	L		0.789	1.37	0.993	0.842
>1.50	L		1.00	1.00	1.00	1.00

BLQ values replaced by ½ BLQ. Source data in TABLE 13: 2.7, 13: 3.7

Pharmacodynamics: FEV_1 measured following a single dose of tiotropium is shown in Figure 2. Figure 3 shows that mean FEV_1 trough and average response to tiopropium was greater than placebo throughout the 49-week treatment period.

Figure 2. Mean FEV_1 over time.

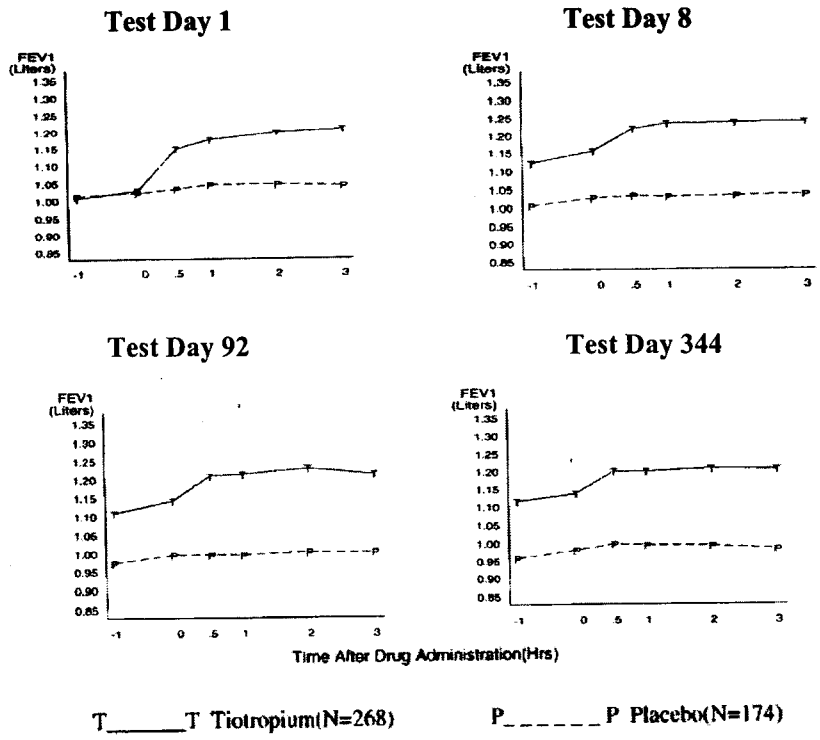
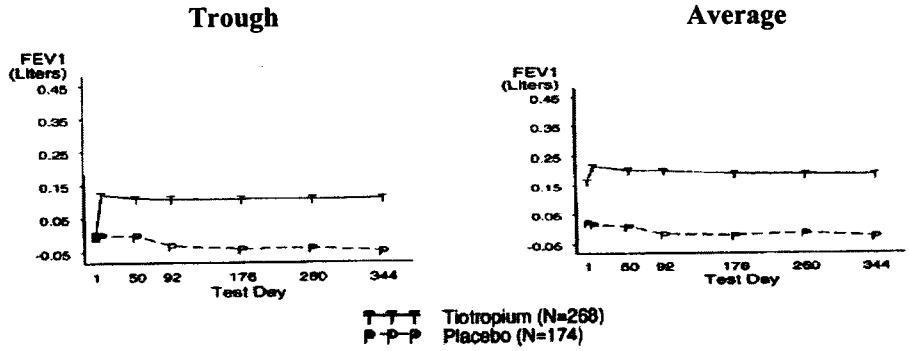


Figure 3. The mean FEV_1 trough and average response over 49-weeks



Summary: (1) Tiotropiuin drug plasma concentrations fluctuate in steady state between ~ 3 pg/mL at trough and 15-18 pg/mL 5 min after inhalation. Drug concentrations decrease rapidly after inhalation and reach 7-8 pg/mL 2 hrs after inhalation. Renal excretion in steady state accounts for 7% of the nominal dose after dry powder inhalation in 24 h, while 0.4% and 1.2-1.3% of the dose are excreted in the 2 hrs before and after inhalation during once daily inhalation. (2) There was a detectable effect of moderate renal dysfunction on tiotropiutn plasma concentrations 2 hrs after inhalation, while maximum drug concentrations were not affected. Gender as well as age (at least as long as it is not associated with relevant renal dysfunction) and lung function had no relevant impact of tiotropium plasma concentrations. Moderate renal dysfunction was combined with a trend to higher tiotropium plasma concentrations. (3) Significant increase in FEV_1 was observed following tiotropium doses.

Protocol 205.120 (Study Report #U94-0198)

Study Type: Single dose PK and PD in COPD

Title: Dose-response and time-response study of Ba 679 BR in patients with chronic obstructive pulmonary disease. A double blind, placebo controlled randomized crossover study assessing the efficacy and safety of Ba 679 BR following single inhalation of 10, 20, 40 and 80 µg on separate days.

Clinical Investigators: [redacted]

Objectives: To evaluate the dose-dependent bronchodilator properties of tiotropium in patients with chronic obstructive pulmonary disease. Some plasma and urine samples were collected to evaluate the preliminary PK of tiotropium in COPD patients (Phase II study).

Study design: Single dose double-blind crossover dose-ranging study using powder capsules. In this trial patients inhaled single doses of tiotropium (8.8 –70.4 µg) and matching placebo, formulated (Phase I formulation) in a lactose based powder capsule and delivered by the FO2 device. The washout period between each test drug inhalation was 72 hours. A total of 35 patients were enrolled (32 male, 3 female; mean age 64 years and FEV_1 of 1.34 liters).

Formulations: Tiotropium bromide monohydrate (Ba 679 BR) was administered as lactose inhalation capsules corresponding to

- 8.8 µg tiotropium (= 11 µg Ba 679 BR, batch 203001, 23% < [redacted] or
- 17.6 µg tiotropium (= 22 µg Ba 679 BR, batch 203002, 22% < [redacted] or
- 35.2 µg tiotropium (= 44 µg Ba 679 BR, batch 203003, 29% < [redacted] or
- 70.4 µg tiotropium (= 88 µg Ba 679 BR, batch 203004, 29% < [redacted] or
- placebo inhalation capsules (batch 204001)

Breath was held as long as it was comfortable after inhalation. Inhalation was done twice from each capsule in order to empty the capsule completely.

Sampling times: Plasma samples were collected 5 minutes after each inhalation. Urine was collected after each inhalation in fractions 0-4 h, and 4-8 h.

Assays: [redacted] with a limit of quantification of [redacted] for plasma and [redacted] pg/mL for urine samples. Assay precision was within [redacted] and [redacted] for plasma and urine and within [redacted] % and [redacted] for accuracy in plasma and urine, respectively.

Criteria for evaluation:
PK: Tiotropium plasma and urine concentrations.
PD: FEV_1 and secondary pulmonary function variables FVC, PEFR and $FEF_{25-75\%}$.

Results: Tiotropium plasma concentrations were listed for those patients receiving their first tiotropium dose. The sponsor reported that remaining drug concentrations are biased due to the long terminal elimination half-life of tiotropium, therefore, these data were not evaluated. Respective concentrations in urine were used to calculate the amounts excreted unchanged in urine (Ae0-4h , Ae0-8h). The results of plasma and urine data are presented in Table 1. Bronchodilation effect of tiotropium (FEV_1) in intent-to-treat patients is shown in Figure 1.

Tiotropium plasma concentrations 5 minutes after inhalation increased with increasing dose. A comparison with dose-normalized data in young healthy subjects from Studies 205.104 (NC_{5min} 15.1 and 6.87 pg/mL), 205.103 (NC_{5min} 8.61 and 13.7 pg/mL) and 205.105 (NC_{5min} 10.9 pg/mL) showed that COPD patients in this study (NC_{5min} 7.52 - 8.57 pg/mL) tended to have lower tiotropium plasma concentrations 5 minutes after inhalation.

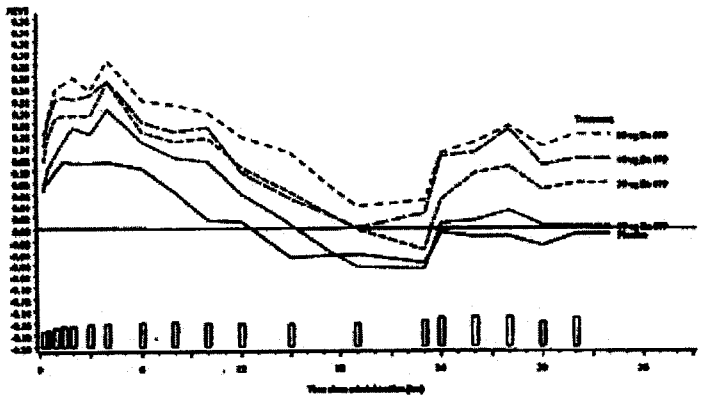
The urinary excretion showed a positive deviation from dose-proportionality with increasing doses.

Table 1. Geometric mean (% gCV) tiotropium plasma concentrations and excretion in urine after a single inhalation of tiotropium by COPD patients.

		n	8.8 µg		17.6 µg		35.2 µg		70.4 µg	
			gMean	%gCV	gMean	%gCV	gMean	%gCV	gMean	%gCV
C_{5min}	[pg/mL]	-/-/7/8	#	--	##	--	14.7	21.3	33.5	55.5
NC_{5min} for 18µg	[pg/mL]	-/-/7/8	#	--	##	--	7.52	21.3	8.57	55.5
Ae_{0-4h}	[% of dose]	7/5/8/8	0.850	89.1	1.50	55.3	1.70	27.3	1.69	25.3
Ae_{0-8h}	[% of dose]	7/5/8/8	1.44	91.9	2.64	48.4	2.83	19.4	2.64	28.0

8 of 10 values < BLQ
4 of 7 values <BLQ Source data in U94-0198

Figure 1. Increase in Mean* FEV_1 (L) from Test-Day Baseline in intent-to-treat patients.



The 2nd peak was seen at around 24-hrs post dose. However, this peak doses not due to pharmacokinetics of tiotropium (i.e., no active metabolites, no enterohepatic recirculation). It could be as the sponsor suggested due to circadian rhythum.

Conclusion:

- Tiotropium plasma concentrations 5 minutes after inhalation tended to be lower in COPD patients in comparison to young healthy subjects.
- The urinary excretion showed a positive deviation from dose-proportionality with increasing doses.

Protocol 205.127 (Study Report #U00-0077)

Study type: Multiple dose PK with inhalation solution via Respimat in COPD patients.

Title of study: Pharmacodynamic and pharmacokinetic dose ranging study of tiotropium bromide administered via Respimat device in patients with chronic obstructive pulmonary disease (COPD): a randomized, 3-week multiple- dose placebo controlled, intraformulation double-blind study, parallel group study.

Coordinating investigator: [redacted].

Objectives: To determine the optimal dose of tiotropium inhaled as a solution using a Respimat device once a day for 3 weeks in patients with COPD. Some urine samples were collected to evaluate the urinary excretion of tiotropium after various Respimat doses in comparison to dry powder inhalations (as reference comparator).

Formulations and posology: Tiotropium was administered as tiotropium bromide monohydrate as lactose inhalation capsules (via HandiHaler) and as solution dispersed by the Respimat device.

- 18.0 µg tiotropium (batch 9706007, 18% <5.8 µm) or
- placebo inhalation capsules (batch 9611002) or solution by the Respimat device
- two puffs of 0.625 µg tiotropium (= 1.25 µg) once daily (batch 9707206) or
- two puffs of 1.25 µg tiotropium (= 2.5 µg) once daily (batch 9707207) or
- two puffs of 2.5 µg tiotropium (= 5.0 µg) once daily (batch 9708201) or
- two puffs of 5.0 µg tiotropium (= 10 µg) once daily (batch 9708202) or
- two puffs of 10 µg tiotropium (= 20 µg) once daily (batch 9708203) or
- two puffs of placebo once daily (batch 9707202)

Study design: Randomized double-blind, placebo controlled, parallel group comparison study evaluating the bronchodilative response of tiotropium by spirometry in COPD patients ≥ 40 years of age Phase II). The total study population consisted of 202 patients (173 male and 29 female; mean age = 60.2 yr with range 38-83 yr).

Criteria for Evaluation: Urine was collected during two-hour fractions predose and post dose on Days 7, 14 and 21.

Analytical Methodology:
Methods: Urine samples were assayed by [redacted] assay with a limit of quantification of
Precision and Accuracy: Precision in urine samples was within [redacted] for precision and [redacted] for accuracy, respectively.

Data analysis (PK): Respective concentrations in urine were used to calculate the amounts excreted unchanged in urine (Ae_{2-0h}, Ae_{0-2h}).

Results: data are summarized in Table 1 and 2. Geometric mean values of urinary excretion values are plotted in Figure 1.

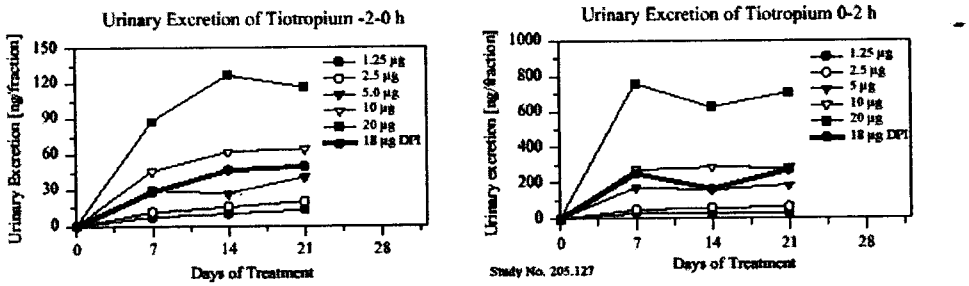
The urinary excretion of tiotropium showed an increase with increasing doses in both predose and postdose samples. Postdose data for Day 7 were very similar to those obtained after 21 days, however, predose values increased from Day 7 to Day 21 as expected from the long elimination half-life of the drug (therefore, the steady state has not reached on Day 7. The sponsor expressed as 'approximate steady state has reached on Day 7').

Table 1. *Comparison of geometric mean tiotropium excreted in urine (ng) in COPD patients.*

Dose (mcg)	Day 7 ± 2		Day 14 ± 2		Day 21 ± 2	
	2-h pre-dose gMean (% gCV)	2-h post-dose gMean (% gCV)	2-h pre-dose gMean (% gCV)	2-h post-dose gMean (% gCV)	2-h pre-dose gMean (% gCV)	2-h post-dose gMean (% gCV)
1.25 N = 10	7.38 (50.4)	29.4 (64.9)	10.2 (82.1)	28.2 (88.1)	13.2 (79.8)	31.1 (106)
2.5 N = 9	N = 8 11.5 (43.3)	47.3 (100)	27.6 (118)	53.2 (82.8)	23.4 (62.0)	50.9 (107)
5.0 N = 10	29.9 (51.2)	170 (60.1)	35.4 (144)	167 (66.3)	48.0 (66.6)	185 (50.3)
10 N = 12	45.8 (97.9)	273 (59.5)	N = 11 62.3 (54.1)	N = 10 241 (90.0)	N = 11 74.1 (68.4)	N = 11 283 (54.7)
20 N = 11	87.5 (88.4)	759 (75.0)	164 (144)	N = 10 690 (98.4)	117 (135)	706 (104)
DRY POWDER INHALATION						
18 N = 9	28.2 (83.4)	251 (63.2)	N = 8 46.2 (58.5)	N = 8 124 (121)	N = 8 45.9 (86.6)	N = 8 192 (140)

The Figure 1 indicates that a tiotropium dose of 5 µg to 10 µg given by the Respimat is equivalent to a 18 µg dose given by the HandiHaler, at least in terms of urinary excretion.

Figure 1. Geometric mean tiotropium excretion in urine (ng) in COPD patients after various tiotropium doses



Conclusions:

- The urinary excretion of tiotropium increased with increasing doses when given by Respimat.
- Doses of 5 to 10 µg tiotropium by Respimat were equivalent to 18 µg tiotropium given by the HandiHaler based on urinary excretion data.

***Protocol 205.133** (Study Report #U00-3029)*

Study type: PK in elderly COPD patients, multiple dose.
Title of study: The pharmacokinetics, safety and tolerability of tiotropium in elderly COPD patients (open label).
Investigator: [redacted]
Objectives: To evaluate the PK of tiotropium following 14 days of administration (18 µg once daily) in elderly COPD patients.
Study Design: Group comparison in two different groups of male and female COPD patients with an age ≤ 58 years and ≥ 70 years.
Subjects: Twenty five Caucasian COPD patients participated in this study. Mean demographic data for the two groups are given in the table below.

		younger patients 5 male, 7 female		elderly patients 9 male, 4 female	
		mean	range	mean	range
Age	[yr]	53	45-58	74	69-80
Weight	[kg]	67.9	50.0-94.0	66.0	43.0-98.0
Height	[cm]	166	155-175	167	157-178
CL_{cr}	[mL/min]	90	51-122	61	47-87

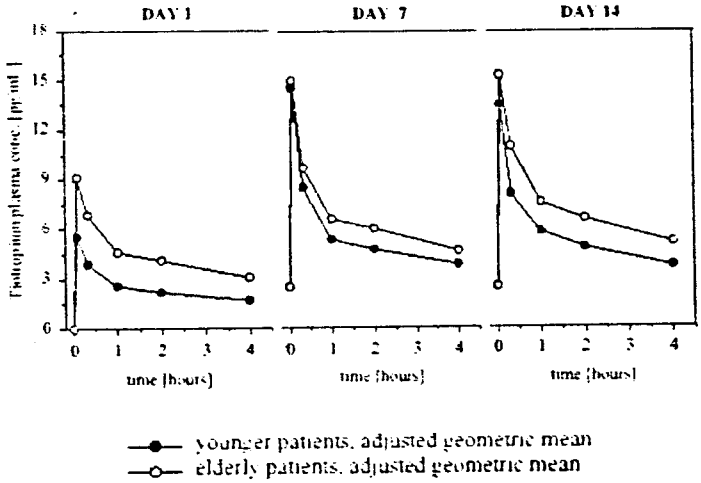
CL_{cr}: creatinine clearance calculated from serum creatinine by the Cockroft-Gault formula

Formulations: Tiotropium was administered as once daily dry powder inhalation of one 18 µg tiotropium lactose inhalation capsule (batch 9706007 = PD-1824, 18% <5.8 µm) by aid of the HandiHaler device. Breath was held as long as it was comfortable after inhalation. Inhalation was done twice from each capsule in order to empty the capsule completely. There were no dietary restrictions with the exception of that no methylxanthine (caffeine etc) containing food or beverages was allowed on the morning of Days 1, 7 and 14. Phase 3 formulation was used in this study.
Analytical Methodology:
Assay Method: HPLC [redacted] with a limit of quantification of [redacted] tiotropium cation in plasma and [redacted] g/mL urine.
Accuracy and Precision: Assay precision in plasma samples was within [redacted] and assay accuracy was within ± [redacted] Respective values for urine samples were [redacted] for precision and [redacted] for accuracy.
Sampling times: Plasma was collected before and 5, 20 minutes, 1, 2 and 4 hours after inhalation on Days 1, 7 and 14. Urine was collected in fractions 0-4 h post dose after the doses on Days 1, 7, 14, 15, 16, 20, 24, 28, 31, 35 and 38 to monitor the tiotropium washout in urine.
Criteria for evaluation: C_{max}, AUC_{0-4h}, $t_{1/2}$ and amount of drug excreted unchanged in urine (Ae).
Data analysis: Tiotropium plasma concentrations below the limit of quantification were replaced by ½ the limit of quantification values in order to fit the plasma data to a model (4-compartment model). Renal clearance was calculated from the amount excreted unchanged in urine divided by the respective AUC for this interval. Apparent terminal elimination rate constants (λ_z) and the terminal elimination half-life was calculated from the log-linear urinary excretion rates.

Results: Geometric PK parameters and geometric mean plasma concentration time-profiles of tiotropium are presented in Table 1 and Figure 1, respectively.

58% of all tiotropium plasma concentrations were below the limit of quantification (mostly Day 1 samples), while urine yield no analytical problems.

Figure 1. Geometric mean tiotropium plasma concentration time-profiles after once daily dry powder inhalations of 18 µg tiotropium for 14 days to younger and older COPD patients.



Plasma: There was no obvious difference in C_{5min} or AUC values between Days 7 and 14 and thus steady state is considered to be achieved within 7 days for practical purposes. C_{5min} values tended to be lower (4.87-7.06 pg/mL) on Day 1 for COPD patients of this study than dose-normalized C_{5min} values for young healthy subjects Studies 205.103 (8.61-13.7 pg/mL), 205.104 (6.9-15.1 pg/mL) or 205.105 (10.9 pg/mL). The difference between healthy subjects and COPD patients was still present on Day 14 with 9.63 pg/mL and 15.3 pg/mL in this study compared to 16.5 pg/mL and 25.1 pg/mL in Study 205.104. Other COPD patients in Study 205.117 showed C_{5min} values between of 14.5 and 18.0 pg/mL, when values below the limit of quantification were replaced as in this study. The sponsor suggested that the reasons for the lower drug plasma concentrations in this study could be as follows: (1) a lower lung function in COPD patients. (2) It might be that the inhalation capsules in Studies 205.133 and 205.117 showed fraction of 18% and 22% <5.8 µm, while those for healthy subjects in Studies 205.103 (29, 29%), 205.104 (23, 22, 29%) and 205.105 (27%) varied from 18-29%. Note: Studies 205.103 and 104 = Phase I formulation. Studies 205. 105/117.133 = Phase III formulation.

Urine: Renal clearance was lower in the older patients (163 *vs.* 326 mL/min), which is a result of decreased urinary excretion (1.42 *vs.* 1.97% of dose on Day 14) in combination with moderately increased AUC_{0-4h} values (26.1 *vs.* 18.2 pg.h/mL). Renal tiotropium clearance is consequently also decreasing from 486 mL/min in young subjects (Study 205.105) over 326 mL/min in intermediately old to 163 mL/min in old COPD patients. Tiotropium is excreted by active secretion process, therefore, decrese of renal excretion of tiotropium as increasing age is expected (i.e., decrease in renal fuction with old age).

Table 1. Geometric mean tiotropium PK parameters in younger and older COPD patients.

		younger patients (n=12)		older patients n=13)	
		gMean	%gCV	gMean	%gCV
Single dose data, Day 1					
C_{5min}	[pg/mL]	4.87	68.7	7.06	82.6
AUC_{0-2h}	[pg.h/mL]	5.99	27.5	8.12	46.1
AUC_{0-4h}	[pg.h/mL]	11.2	19.5	13.7	30.8
Ae_{0-4h}	[% of dose]	0.606	143	0.661	65.8
Multiple dose data, Day 7					
C_{5min}	[pg/mL]	11.6	116	13.2	85.3
AUC_{0-2h}	[pg.h/mL]	10.9	72.6	13.7	60.9
AUC_{0-4h}	[pg.h/mL]	17.9	58.9	21.8	49.4
Ae_{0-4h}	[% of dose]	1.61	108	1.42	48.6
CL_r	[mL/min]	268	82.3	194	65.4
Multiple dose data, Day 14					
C_{5min}	[pg/mL]	9.63	142	15.3	60.0
AUC_{0-2h}	[pg.h/mL]	10.8	83.4	15.7	66.8
AUC_{0-4h}	[pg.h/mL]	18.2	69.2	26.1	62.5
Ae_{0-4h}	[% of dose]	1.97	74.4	1.42	88.7
CL_r	[mL/min]	326	60.2	163	92.8
$t_{1/2}$	[h]	132	28.9	156	28.7
$t_{1/2}$	[days]	5.5	28.9	6.5	28.8
Model dependent evaluation (n=9 for younger and n=11 for older patients)					
C_{max}	[pg/mL]	12.2	90.8	13.9	61.6
t_{max}	[h]	0.042	97.7	0.066	80.8
$AUC_{0-\infty}$	[pg.h/mL]	132	38.2	141	25.9
CL/f	[mL/min]	# 2270	38.2	# 2123	25.9
V_{ss}/f	[L]	# 11310	75.1	# 10420	45.3
MRT_{tot}	[h]	84.0	56.7	82.3	46.9
partial AUC	% of total AUC, b4	45.6	71.5	35.0	97.7
$t_{1/2}$	[h] b3	7.9	119	9.5	107
$t_{1/2}$	[h] terminal, b4	108	33.5	131	42.9
$t_{1/2}$	[days] terminal, b4	4.5	33.5	5.5	42.9

the absolute bioavailability of 19.5% (cf 205.105/U99-1315) influenced these values. To compare them to an intravenous dose like in 205.105 they should be consequently divided by a factor of ~5 (100/~18)

§ median and range, source data in U00-3029

Conclusions: C_{5min} of tiotropium was 45, 14 and 59% higher in the elderly than the younger COPD patients on Day 1, 7 and 14, respectively, and associated with lower amount of tiotropium excreted unchanged in the urine. Renal tiotropium clearance was significantly lower in the elderly patients (163 mL/min) compared with the young patients (326 mL/min). Elimination half-life was about 20% longer in the elderly compared to that of in the younger COPD patients (6.5 days in the elderly, 5.5 days in the young group).

Protocol 205.134 *(Study Report #U00-1289)*

Study type: PK in subjects with renal impairment after iv dose.

Title of study: The pharmacokinetics, safety and tolerability of tiotropium (4.8 µg single iv dose) in outpatients with renal impairment in comparison to healthy subjects.

Investigator: —

Objectives: To investigate the PK of a single intravenous dose of tiotropium (4.8 µg) in patients with renal impairment in comparison to healthy volunteers.

Study Design: PK of tiotropium was compared in four different groups of male and female patients with normal to severe renal impairment.
Subjects: 24 Caucasian subjects participated in this study. Mean demographic data for the 4 groups are given in the table below.

		normal CL_{CR} >80 mL/min		mild impairment CL_{CR} >50-80 mL/min		moderate impairment CL_{CR} >30-50 mL/min		severe impairment CL_{CR} <30 mL/min	
		n=6	6 m, 0 f	n=5	5 m, 0 f	n=7	6 m, 1 f	n=6	4 m, 2 f
		mean	range	mean	range	mean	range	mean	range
Age	[yr]	51.7	39-60	55.2	36-63	55.1	40-64	48.5	36-58
Weight	[kg]	76.5	59-95	83.7	71-96	82.4	64-99	68.5	50-82
Height	[cm]	176	169-182	174	164-183	178	169-189	172	157-184
CL_{CR}	[mL/min]	108	96-126	70.4	63-77	44.1	40-49	23.5	18-29

CL_{CR}: creatinine clearance calculated from serum and urine creatinine

Formulations: Tiotropium was administered as an intravenous infusion of tiotropium bromide monohydrate (Ba 679 BR) over 15 minutes. Tiotropium solution provided in ampoules containing 48 µg tiotropium (60 µg Ba 679 BR) in 1.0 mL (batch 9707203) was diluted 1: 10 and 10 mL diluted solution was infused.

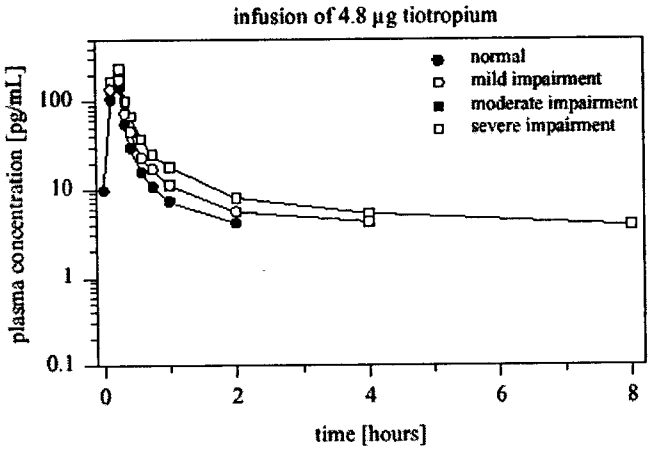
Analytical Methodology:
Assay Method: HPLC- — with a limit of quantification of — pg/mL tiotropium in plasma and — pg/mL in urine.
Accuracy and Precision: Assay precision in plasma samples was within —, and assay accuracy was within ± —. Respective values for urine samples were — for precision and — % for accuracy.

Sampling times: Plasma was collected before and 7, 15, 20, 25, 35, 45 minutes and 1, 2, 4 and 8 hours after start of infusion. Urine was collected in fractions 0-4 h, 4-8 h, 24-28 h, 28-32 h, 48-52 h, 52-56 h, 144-148 h, 148-152 h, 240-244 h, 244-248 h, 336-340 h, 408-412 h, 412-416 h, 504-508 h, 508-512 h, 576-580 h, 580-584 h.

Criteria for evaluation: C_{max}, AUC_{0-4h}, $t_{1/2}$, CLr, and amount of drug excreted unchanged in urine (Ae).

Results: *Geometric PK parameters and geometric mean plasma concentration time-profiles of* tiotropium are presented in Table 1 and Figure 1, respectively.

Figure 1. Geometric mean tiotropium plasma concentration time-profiles after intravenous infusion of 4.8 µg tiotropium to subjects with varying degrees of renal impairment.



Plots of individual pharmacokinetic parameter values versus measured CLcr values are shown in Figure 2.

Figure 2. PK parameters *vs.* CLcr.

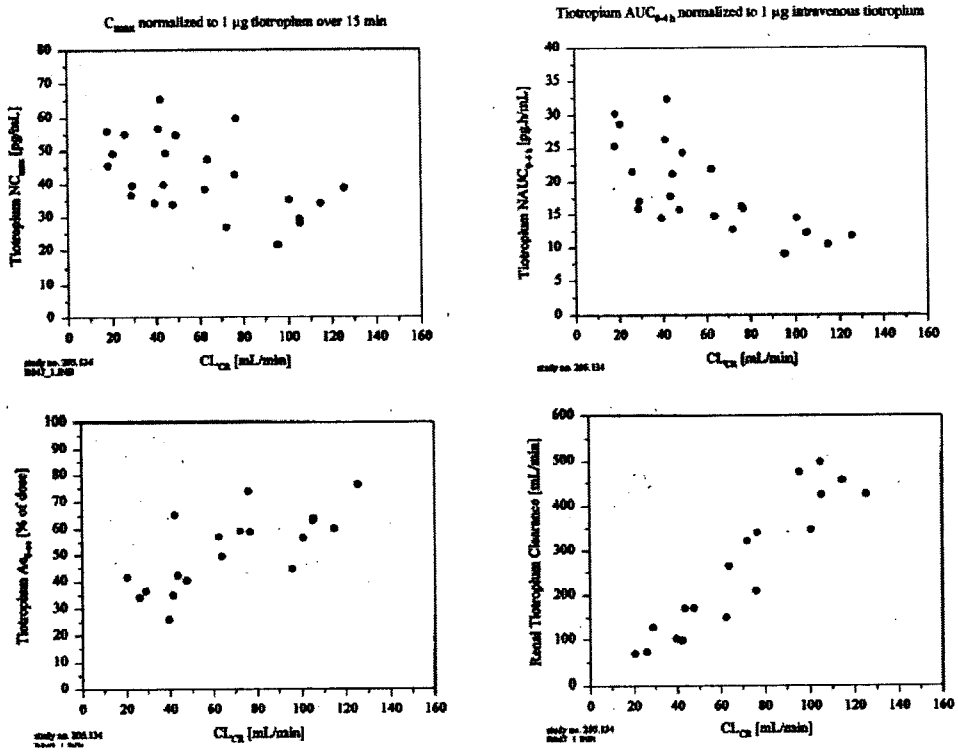


Table 1. Geometric mean (% gCV) tiotropium pharmacokinetic parameters after intravenous infusion of 4.8 µg tiotropium to subjects with varying degrees of renal impairment.

		severe impairment n=6		moderate impairm. n=7		mild impairment n=5		normal n=6	
		CL_{cr} <30 mL/minCLCR		30-50>				80 mL/min	
		gMean	%gCV	gMean	% gCV	gMean	% gCV	gMean	% gCV
C_{max}	[pg/mL]	223	17.5	223	26.5	200	30.1	147	21.3
AUC_{0-4h}	[pg.h/mL]	108	27.3	101	29.8	77.1	20.1	55.5	16.2
Ae_{0-4h}	[% of dose]	11.0 *	14.6	15.1	31.4	23.7 #	20.1	30.2	11.4
$Ae_{0-\infty}$	[% of dose]	37.4 *	10.2	39.9	34.5	59.3 #	14.4	60.1	17.7
CL_r	[mL/min]	85.7 *	35.5	124	29.9	246 #	34.8	435	12.7
$t_{1/2}$	[days]	5.95 *	29.3	3.96	32.3	5.02 #	45.1	4.03	19.1

*: n=3, #: n=5

Maximum drug plasma concentrations as well as AUC values tended to increase with the degree of renal impairment.

The terminal elimination half-life did not change with the deterioration of renal function. This couyld be due to analytical limitation (i.e., urinary excretion could not often be measured after 200-300 hrs due to low tiopropium concentrations, which means an observation for about 2 elimination half-lives instead of the desired 5 elimination half-lives).

Conclusions:

- Tiotropium plasma concentrations increased with renal dysfunction with more pronounce changes in subjects with a CLcr < 50 mL/min (AUC_{0-4h}: +39, +81 and +94% from normal to mild, moderate and severe renal dysfunction).
- Urinary excretion and renal clearance decreased along with renal function.

Protocol 205.201 (Study Report #U98-3174)

Study type: Multiple dose PK in asthma patients.

Title of study: The effect of twenty-one day dosing of tiotropium on bronchomotor tone in patients with moderate to severe asthma. A randomized double blind placebo controlled parallel study.

Principal investigator: [redacted]

Objectives: To evaluate the bronchodilator properties of tiotropium in moderate to severe asthmatics, preliminary PK and safety. Urine was collected to evaluate compliance and to obtain some PK data in asthma patients.

Formulations and posology: Tiotropium was administered as tiotropium bromide monohydrate (Ba 679 BR) once daily for three weeks as lactose inhalation capsules corresponding to

- 1 x 4.5 µg tiotropium (batch PD-1798 = 9611004, 20% [redacted]) or
- 1 x 9.0 µg tiotropium (batch PD-1790 = 9611008, 18% [redacted] or
- 1 x 18.0 µg tiotropium (batch PD-1791 = 9602003, 17% [redacted] or
- 1 x 36.0 µg tiotropium (batch PD-1792 = 9602005, 28% [redacted]
- placebo (9602001)

The inhalation capsules (Phase 3 formulation) were administered in the morning by aid of the HandiHaler Device.

Study design: Randomized double blind placebo controlled parallel group comparison study evaluating the bronchodilative response of tiotropium by spirometry in asthma patients. There were no plasma collections for tiotropium in this trial. Urine was collected in a subset of patients (13 centers with a total number of subjects of 180) on Day 1, 7, 14 and 21 in fractions of 2 hours before (-2-0 h) and 2 hours after (0-2 h) the inhalation. Primary efficacy endpoints was an improvement in FEV_1.

Subjects: Evaluable urine concentrations were obtained from 126 patients (61 females, 65 males; mean age of 39.1 with range 20-75 years), the total study population consisted of 204 patients.

Analytical Methodology:

Assay Method: HPLC [redacted] with a LOQ of [redacted] pg/mL tiotropium cation.

Accuracy and Precision: Assay precision in urine samples was within [redacted] for precision and [redacted] for inaccuracy.

Data analysis: Tiotropium concentrations in urine were used to calculate the amounts excreted unchanged in urine ($Ae_{0\text{-}2h}$, $Ae_{\text{-}2\text{-}0\,h}$).

Results: Data from 3 centers (13-15) were excluded from the PK evaluation due to the apparent mislabeling of urine samples (urine samples from patients who received 36 µg from these centers were BLQ, while samples from placebo according to the randomization schedule showed high concentrations in their urine). PK data are summarized in Table 1.

Table 1. *Geometric mean (% gCV) tiotropium excretion in urine (% of dose) after inhalation* doses of tiotropium for 21 days by asthma patients.

N = 31 - 37	4.5 µg		9 µg		18 µg		36 µg	
	gMean	% gCV	gMean	% gCV	gMean	% gCV	gMean	% gCV
day 1 Ae_{0-2h} (% of dose)	0.525	87.0	0.664	52.8	0.525	66.0	0.924	105
day 7 Ae_{0-2h} (% of dose)	0.875	75.6	1.34	74.8	1.02	72.8	2.04	78.0
day 14 Ae_{0-2h} (% of dose)	1.00	73.1	1.25	100	1.11	60.5	2.23	72.7
day 21 Ae_{0-2h} (% of dose)	1.15	55.3	1.36	82.7	1.19	122	2.15	61.3
day 7 Ae_{-2-0h} (% of dose)	0.547	91.2	0.679	110	0.411	136	0.783	196
day 14 Ae_{-2-0h} (% of dose)	0.583	69.6	0.938	118	0.513	142	0.973	109
day 21 Ae_{-2-0h} (% of dose)	0.673	122	0.988	95.3	0.567	131	1.00	83.7

Urinary excretion data following inhalation showed high inter/intra individual variability (ranged 53-196%). No systemic deviation from proportionality was observed with the dose groups 4.5-18 µg, while 36 µg showed a tendency to a more than proportional increase of the urinary excretion. According to the sponsor this may be due to different inhalation capsule formulation (i.e., had the highest fraction, 28%, of small particles and therefore, highest 'delivered dose'). It appears that "true" steady state has not reached by day 14 (see 0-2 h and -2-0 h data), however, deviation from steady state (day 21) is relatively small.

Impact of Demographic Factors

Impact of demographic factors were investigated using data from day 14 and 21 (represents steady state).

Note: Geometric means (gMean), shown in the tables below, is expressed as % of dose excreted in urine.

A. Impact of ethnicity: No impact of ethnic origin can be concluded, however, the majority of subjects were Caucasians.

ethnic	without centers 13 - 15 and without dose 36 µg (A)				centers 13 - 15, dose 36 µg and unlikely values excluded (B)			
	negroid		caucasian		negroid		caucasian	
	mean day 14 & 21		mean day 14 & 21		mean day 14 & 21		mean day 14 & 21	
	Ae_{-2-0h}	Ae_{0-2h}	Ae_{-2-0h}	Ae_{0-2h}	Ae_{-2-0h}	Ae_{0-2h}	Ae_{-2-0h}	Ae_{0-2h}
N	9	9	95	95	7	8	80	80
gMean	0.587	1.32	0.704	1.18	0.652	1.43	0.590	1.42
gCV (%)	218	92.1	103	71.4	82.3	57.1	57.4	41.6

bb205_201_bl1.xls [ethnic_sort_MW]
bb205_201_methodb.xls [method_b_ethnic]

B. Impact of Gender: No impact of gender can be concluded from the data.

gender	without centers 13 - 15 and without dose 36 µg (A)				centers 13 - 15, dose 36 µg and unlikely values excluded (B)			
	female		male		female		male	
	mean day 14 & 21		mean day 14 & 21		mean day 14 & 21		mean day 14 & 21	
	Ae_{-2-0h}	Ae_{0-2h}	Ae_{-2-0h}	Ae_{0-2h}	Ae_{-2-0h}	Ae_{0-2h}	Ae_{-2-0h}	Ae_{0-2h}
N	58	58	47	47	43	43	45	46
gMean	0.671	1.09	0.713	1.30	0.571	1.35	0.613	1.46
gCV (%)	142	90.7	72.7	47.0	66.1	48.4	52.0	39.0

bb205_201_bl1.xls [gender_sort_MW]
bb205_201_methodb.xls [method_b_gender]

C. Impact of Age: *The age distribution in this study ranged from 20 to 75 years with a mean of* 39 years, and analysis results are shown in the tables below:

age	without centers 13 - 15 and without dose 36 µg					
	< 55 years mean day 14 & 21		55 - 65 years mean day 14 & 21		> 65 years mean day 14 & 21	
	Ae_{-2-0h}	Ae_{0-2h}	Ae_{-2-0h}	Ae_{0-2h}	Ae_{-2-0h}	Ae_{0-2h}
N	93	93	8	8	4	4
gMean	0.693	1.21	0.705	0.884	0.574	1.34
gCV (%)	115	73.2	78.7	86.0	59.9	10.0

bb205_201_bll.xls [age_port_MW]

Urinary excretion decreases slightly with increasing age in average in the pre-dose fraction ($Ae_{-2-0\,h}$) where as no trend is obvious in the fraction after inhalation ($Ae_{0-2\,h}$). No general conclusion can be drawn for impact of age due to the low number of patients who are older than 55 years.

D. Impact of creatine clearance: No impact of CLcr can be concluded from the data (none of the subjects had CLcr < 50 mL/min).

CL_{CR}	without centers 13 - 15 and without dose 36 µg			
	50 - 80 ml/min mean day 14 & 21		> 80 ml/min mean day 14 & 21	
	Ae_{-2-0h}	Ae_{0-2h}	Ae_{-2-0h}	Ae_{0-2h}
N	29	29	76	76
gMean	0.673	1.31	0.695	1.14
gCV (%)	97.2	67.9	116	74.5

E. Effect of FEV_1 on urinary excretion: Summary table of FEV_1 data on urinary excretion are presented in table below.

method A $FEV\,1_{pre}$ [L]		day 1 $FEV\,1_{pre}$ [L]	mean d14&21 Ae_{-2-0h} (% of dose)	Ae_{0-2h} (% of dose)
female	N	30	29	29
< median 1.97	gMean	1.63	0.511	0.988
	gCV (%)	13.3	85.0	81.2
female	N	30	29	29
> median 1.97	gMean	2.20	0.881	1.21
	gCV (%)	8.80	192	100
male	N	25	22	22
< median 2.38	gMean	1.91	0.653	1.33
	gCV (%)	15.9	67.1	50.5
male	N	26	25	25
> median 2.38	gMean	2.85	0.769	1.28
	gCV (%)	8.93	77.9	44.9

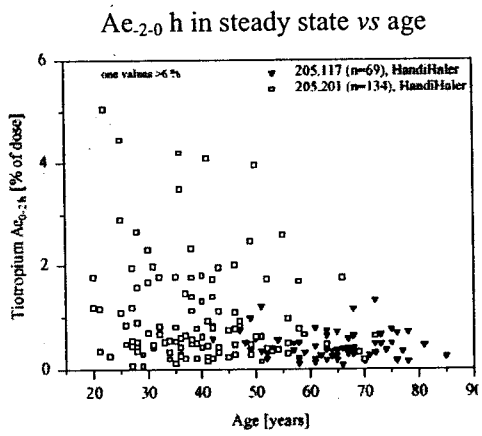
It is evident that urinary excretion is reduced by about 70 % in females having low FEV_1 values in the pre-dose fraction. In males there is only a small difference (~10%). Thus, it could be possible that FEV_1 values <1.97 L affects pharmacokinetics of tiotropium through lower absorption (i.e., lower absorption which leads to lower elimination).

Comparison of PK to a similar study in COPD patients, 205.117

G. Impact of Age: Age distribution of < 55, 55-65 and >65 years in study 205.117 were 16, 28 and 34 patients, while those were 93, 8 and 4 subjects in 205.201. Thus, younger patients were participated in this study compared to that in COPD study. Urinary excretion for different age groups is shown in Table below, and geometric mean values are plotted in Figure 1 below.

	205.117	205.201	205.117	205.201	205.117	205.201
age	% excr. -2 - 0 h	% excr. -2 - 0 h	% excr. 0 - 2 h	% excr. 0 - 2 h	ratio +2 h/-2 h	ratio +2 h/-2 h
<55	0.46	0.62	1.73	1.44	3.75	2.37
55 - 65	0.39	0.54	1.27	1.26	3.29	2.34
>65	0.42	0.38	1.26	1.42	3.24	3.76
all	0.41	0.60	1.35	1.43	3.36	2.42

Figure 1. Effects of age on steady state urinary excretion of tiotropium in comparison of asthma and COPD patients (Studies 205.201 and 205.117)



Overall post inhalation urinary excretion ($Ae_{0\text{-}2hr}$) was similar, and it may suggest similar extent of absorption. Pre-dose excretion ($Ae_{-0\text{-}2hr}$) was somewhat higher in the younger asthma patients (suggests elimination is higher) compared to that in COPD patients. However, conclusive differences between the groups can not be made due to uneven subject numbers.

H. Impact of FEV_1 values: Median values of female and male patients before starting treatment in 205.117 were 1.04 and 1.12 L, respectively and corresponding values in this study were 1.96 and 2.36 L, respectively. Thus, it is evident that COPD patients had much lower FEV_1 values than asthma patients. Urinary excretion for different sub panels is given in table below:

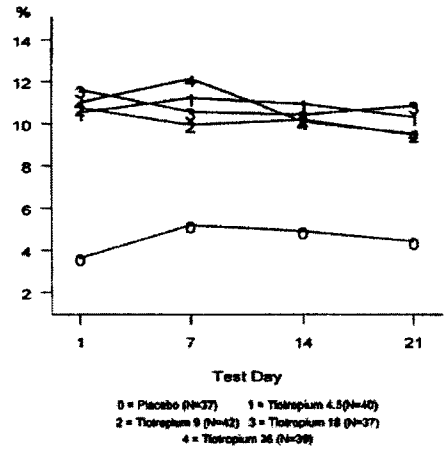
	205.117	205.201	205.117	205.201	205.117	205.201
panel (FEV_1-vol)	% excr. -2 - 0 h	% excr. -2 - 0 h	% excr. 0 - 2 h	% excr. 0 - 2 h	ratio +2 h/-2 h	ratio +2 h/-2 h
female low	0.39	0.51	1.42	1.08	3.64	2.11
female high	0.35	0.87	1.26	1.44	4.00	1.66
male low	0.41	0.63	1.34	1.30	3.46	2.05
male high	0.47	0.80	1.37	1.30	2.91	1.64
gmean all	0.41	0.69	1.35	1.28	3.36	1.85

$Ae_{0\text{-}2hr}$ was quite similar in COPD patients compared to asthma patients, indicating similar extent of absorption. Pre-dose excretion in 205.117 was lower.

Pharmacodynamics: Changes (from the baseline) in FEV_1 is evaluated as the % of the predicted FEV_1 ("% predicted). The $AUC_{0\text{-}4h}$ for % predicted FEV_1 is depicted in Figure 2. A statistically significant ($p < 0.05$) increase in $AUC_{0\text{-}4h}$ for % predicted FEV_1, was detected as compared to placebo after administration of all four doses of tiotropium. However, dose dependent response

was not observed (i.e., all 4 dosing levels, 4.5-36 µg, of tiopropium showed approximately the same response in terms of FEV_1).

Figure 2. Mean AUC_{0-4h} as a % Predicted FEV_1 response over 21 days



Summary (PK):

- Geometric means of % urinary excretion of doses 4.5 – 18 µg were about 1.4 % for 0 - 2 h and 0.8% for -2 – 0 h, indicating rapid absorption and excretion. Steady state was not reached until Day 21 (increase of urinary excretion was high between days 1 and 7 but still existent from days 7 to 14 and 14- 21).
- A slight deviation from dose-proportionality was observed for the 36 µg inhalation capsules.
- There was no relevant impact of ethnic origin on absorption or excretion of tiotropium, however, most of the subjects were Caucasians (85% Caucasians vs. 13% Blacks).
- There was also no relevant impact of gender (see B) on tiotropium kinetics.
- A trend to a decreased tiotropium excretion with age, however, no solid conclusions on impact of age and/or creatinine clearance can be drawn from this study since none of the subjects had a creatinine clearance <50 mL/mm (see C and D).
- Urinary excretion in females with FEV_1 values <1.97 L in the pre-dose fraction was about 70 % lower than in females with FEV_1 values >1.97 L. In males there is only a small difference of about 10% (see E). Therefore, it appears FEV_1 values <1.97 L affects pharmacokinetics of tiotropium through lower absorption (i.e., lower absorption in which leads to lower elimination).
- Average FEV_1 values in COPD patients (study 205.117) was only 50 % of this study. It is noticed that late urinary excretion ($Ae_{2-0\,h}$) was higher in the asthma patients (about 0.7 %) than in the COPD patients (about 0.4 %), whereas no difference was found in after inhalation, $Ae_{0-2\,h}$ (about 1.4 % in both panels) (see H). Therefore, low FEV1 values did not cause significant differences in terms of absorption.
- A statistically significant increase in FEV_1 was observed with all four doses of tiotropium, however, a dose-response was not demonstrated.

Protocol 205.222 (Study Report #U01-1433)

Study type: DDI

Title of study: The effect of concomitant cimetidine p.o. 400 mg t.i.d. and p.o. ranitidine 300 mg once daily on single dose pharmacokinetics of tiotropium (14.4 pg) given intravenously over 15 minutes in healthy male and female subjects of 50-65 years (randomized, open label, two independent two-fold crossover)

Principal investigator: [redacted] . MD, Germany

Objectives: To investigate the effect of an inhibition of the renal cationic drug transporter on single dose pharmacokinetics of intravenous tiotropium in subjects in an age close to a typical COPD patient population.

Test product: 14.4 µg tiotropium (=18 µg of BA 679 BR) by iv infusion over 15 min (bach no. B000203) and 400mg cimetidine tablets t.i.d. or 300 mg ranitidine tablets once daily for 5 days.

Study design: Open label, randomized, two independent two-fold crossover study in 18 healthy subjects. Two single doses separated by a wash out period of 21 days; Cimetidine and ranitidine: 5 days treatment.

Samples: Blood was collected at t = pre-dose, 7, 15, 20, 25, 35, 45 min and 1, 2, 3, 4 and 8 hrs post dose. Urine was collected in fractions.

Analytical Methodology:
Assay Method: HPLC- [redacted] LOQ for plasma and urine were [redacted] pg/mL tiotropium cation, respectively.
Accuracy and Precision: Assay precision and accuracy for plasma QC samples ranged [redacted] and [redacted] respectively. Corresponding values for urine ranged [redacted] and [redacted] respectively.

Results: PK data are summarized in Table 1.

Table 1. Geometric mean and interindividual % gCV tiotropium PK parameters

		with cimetidine			tiotropium alone			Point	90% CI
Tiotropium 14.4 µg		gMean	%gCV	n	gMean	%gCV	n	estimate	
AUC_{0-4h}	pg.h/mL	304	26.0	6	253	23.3	6	1.20	1.03-1.4
C_{max}	pg/mL	664	26.8	6	635	10.6	6	1.05	0.8-1.37
Ae_{0-96h}	% dose	47.2	15.5	5	48.2	14.8	5	-	-
CLr	mL/min	277	26.4	5	355	19.6	5	-	-
CLcr	mL/min	93.5	25.1	5	102	20.8	5	-	-
		with ranitidine			tiotropium alone			Point	90% CI
Tiotropium 14.4 µg		gMean	%gCV	n	gMean	%gCV	n	estimate	
AUC_{0-4h}	pg.h/mL	254	19.6	12	256	19.4	12	0.99	0.9-1.08
C_{max}	pg/mL	596	24.1	12	683	16.3	12	0.87	0.73-1.04
Ae_{0-96h}	% dose	50.4	11.1	11	50.6	9.7	11	-	-
CLr	mL/min	343	28.0	11	342	25.7	11	-	-
CLr	mL/min	114	21.4	11	110	23.2	11	-	-

Observations in poor metabolizers of cytochrome P450 2D6: There were four poor CYP 2D6 metabolizers identified by genotyping. PK parameters were compared among subjects with different genotypes, and the results are shown in Table 2.

Table 2. Effect of CYP 2D6 genotype on tiotropium PK parameters

	AUC_{0-4h} (pg.h/mL)	Ae_{0-4h} (% of dose)	Ae_{0-24h} (% of dose)
poor metabolizers (n=4)	314 (15.2% gCV) 95% CI (247-399)	40.3 (3.4% gCV)[a] 95% CI (37-43.8)	57.6 (2.1% gCV)[a] 95% CI (54.7-60.7)
extensive metab. (n=15)	237 (17.4% gCV) 95% CI (215-261)	34.4 (12.8% gCV)[b] 95% CI (32-37)	48.4 (9.2% gCV)[b] 95% CI (45.6-50.7)
one defected allel (n=6)	259 (17.2% gCV) 95% CI (217-310)	33.4 (11.3% gCV) 95% CI (29.7-37.6)	49.1 (8.9% gCV) 95% CI (44.7-53.9)
wild type (n=9)	224 (15.8% gCV) 95% CI (199-253)	35.2 (14% gCV)[c] 95% CI (31.3-39.5)	47.5 (9.8% gCV)[c] 95% CI (43.8-51.5)

[a]n=3 [b]n=14 [c]n=8

Conclusions:

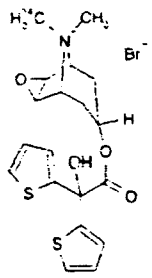
- Geometric mean values of tiotropium AUC_{0-4h} was 20% higher (point estimate 1.2; 90% CI ranged 1.03-1.4) for cimetidine coadministration in comparison to tiotropium alone. C_{max} values did not increase significantly (point estimate 1.05; 90% CI ranged 0.8-1.37). The amount of drug excreted unchanged in urine was slightly less with cimetidine coadministration in comparison with tiotropium alone. Renal clearance (CLr) was reduced by 22% in cimetidine coadministration group in comparison to tiotropium alone group
- Ranitidine did not cause any significant change in PK parameters of tiotropium. .
- AUC_{0-4h} was increased by ~33% in poor 2D6 metabolizers in comparison to extensive metabolizers (overlapping in 95% confidence intervals could be due to small number of subjects). This results showes that CYP 2D6 are involved in metabolism of tiotropium.

In vitro metabolism in human and animal microsomes (U99-1348)

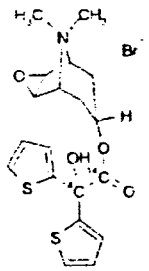
The *in vitro* metabolism of tiotropium bromide was investigated in liver microsomes of rat, dog, mouse and human.

Materials and methods: Investigation was performed with two different radioactively labelled compounds, compound I, labelled in the N- methylscopine moiety and compound II, and labelled in the dithienylglycolic acid moiety. This was done in order to monitor the fate of both parts of the molecule. Incubations with liver microsomes, microsomes containing recombinant human liver CYP isoforms, cytosol, or S9 supernatant were performed in 0.1 M TRIS buffer containing 5 mM magnesium chloride. Metabolites and cleavage products in incubation media were analyzed by HPLC with radiodetection and HPLC-MS/MS.

Compound I



Compound II



Inhibition of CYP 450 catalysed metabolism of tiotropium bromide: Proadifen (SKF 525), an unspecific CYP inhibitor was used to assess whether Ba 679 BR metabolism was mediated by cytochrome P450. Cytochrome P450 catalysed metabolism of Ba 679 in human liver microsomes was investigated by use of furafylline (CYP 1A2), sulphaphenazole (CYP 2C9), gestodene (CYP 3A4), quinidine, (CYP 2D6), and ketoconazole (CYP 3A). These compounds are generally accepted as selective inhibitors of cytochrome P450 isoenzymes in humans. The esterases inhibitors paraoxon, PMSF and PCMB were used to investigate the involvement of microsomal esterases in the formation of N-methylscopine. Also, quercitrin a specific inhibitor of carbonyl reductase was used to trap the keto compound of N-methylscopine, formed intermediately by CPY catalyzed ester cleavage.

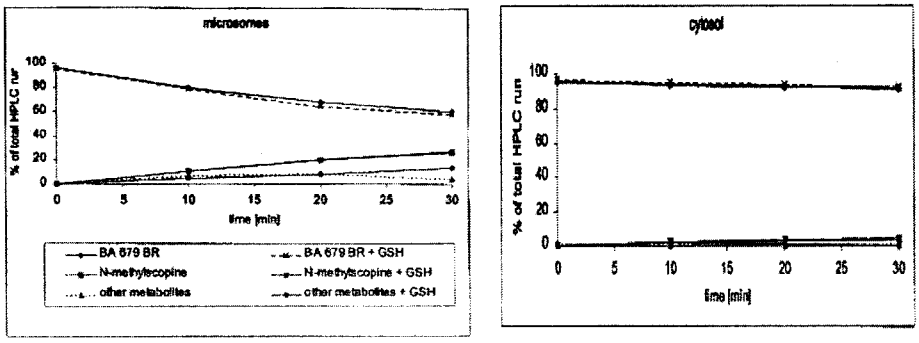
Results: The followings are from the report #99-1348 (Volume 96).

METABOLISM OF BA 679 BR BY LIVER MICROSOMES – QUANTITATION OF METABOLITES USING HPLC

Metabolism of Ba 679 BR by liver microsomes, liver cytosol and liver S9 supernatant of phenobarhital induced rats:

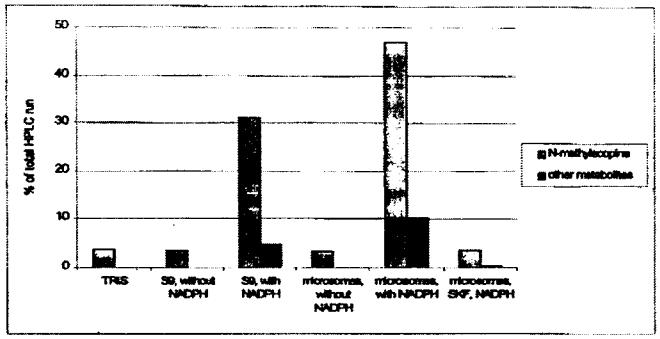
As shown in FIGURE 10:1, in microsomes (and S9 supernatant), the amount of Ba 679 BR (I) decreased and N-methylscopine was formed. Biotransformation of Ba 679 BR was marginal in rat liver cytosol. Glutathinne did not influence the velocity of Ba 679 BR metabolism. Substantial amounts of unidentified metabolites were formed that could not be separated by HPLC.

FIGURE 10: 1 *Metabolism of Ba 679 BR (I) by liver microsomes, liver cytosol and liver S9 supernatant of phenobarbital induced rats.*



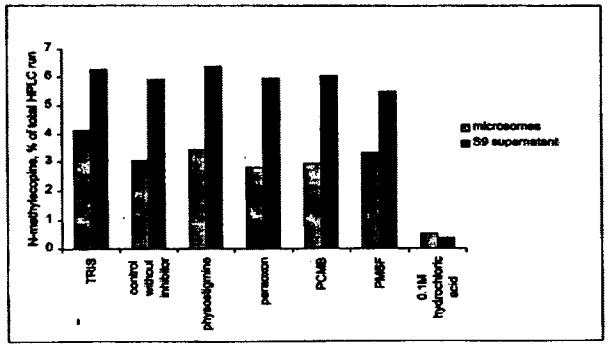
Influence of NADPH and proadifen (SKF 525) on the metabolism of Ba 679 BR by rat liver microsomes: N-methylscopine was formed without NADPH, but "other metabolites" was dependent on NADPH. Proadifen reduced the total metabolism to the value of control incubations in TRIS buffer or without NADPH (FIGURE 10: 3).

FIGURE 10: 3 Influence of NADPH and proadifen (SKF 525) on the metabolism of Ba 679 BR (10 µM, I) by phenobarbital induced rat liver microsomes and S9 supernatant.



Influence of various esterase inhibitors on the metabolism of Ba 679 BR by rat liver microsomes or liver S9 supernatant:

FIGURE 10: 4 **Influence of various esterase inhibitors on the metabolism of Ba 679 BR (10 µM, I) by rat liver microsomes or liver S9 supernatant.**



Esterasc inhibitors did not influence N-methylscopine formation. Addition of hydrochloric acid (0.1 M) to the incubation mixtures was able to prevent N-methylscopine formation (FIGURE 10: 4).

Metabolism of Ba 679 BR by human liver microsomes and liver mierosomes of rat, dog, and mouse, influence of glutathione: As shown in FIGURE 10: 5a, incubations with rat (and mouse) liver microsomes Ba 679 BR disappeared rapidly and N-methylscopine was formed. About 50 % of unidentified metabolites (retention times of 10 to 19 min) were formed. In addition, a polar metabolite fraction was observed that could not be assigned to the metabolite structures of Ba 679 BR metabolites (observed during the *in vivo* studies). Human (and dog) liver microsomes exhibited minute Ba 679 BR metabolism (FIGURE 10: 5a, b) during 2 hrs incubation. Glutathione had only minor influence on the decrease of Ba 679 BR compared to the effect on the formation of metabolites by liver microsomes of rats and humans. N-methylscopine formation was lower, the formation of other metabolites was higher in the presence of GSH. There was no appreciable effect of GSH in experiments with dog and human liver microsomes.

FIGURE 10: 5a **Metabolism of Ba 679 BR (10 µM, I) by human liver microsomes and liver microsomes of rat, dog, and mouse in the presence and absence of glutathione.**

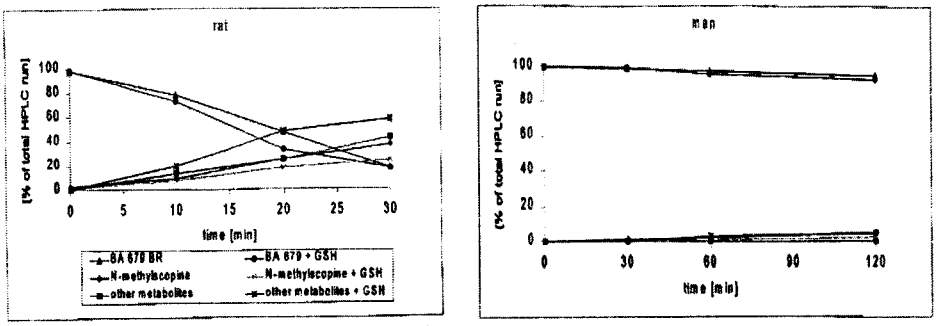
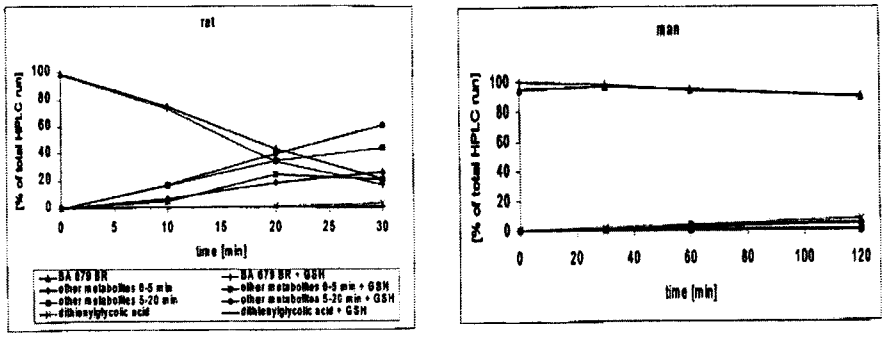


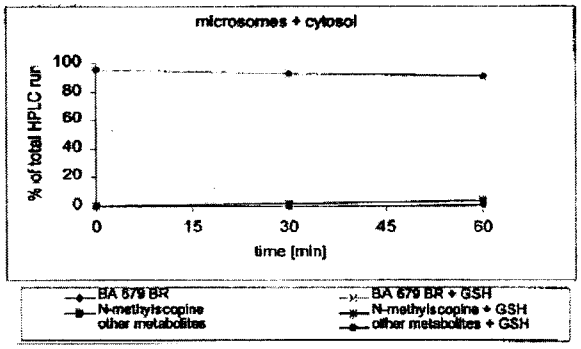
FIGURE 10: 5b shows the result of the identical incubation conditions using Ba 679 BR that was labelled in the dithienylglyeolic acid moiety (10 µM, II). The decrease of Ba 679 BR was comparable to Ba 679 BR. I. Formation of dithienylglycolic acid was lower than N-methylscopine formation in parallel incubations.

FIGURE 10: 5b **Metabolism of Ba 679 BR (10 µM, II) by human liver microsomes and liver microsomes of rat, dog, and mouse in the presence and absence of glutathione.**



Metabolism of Ba 679 BR by human liver microsomes and human liver cytosol - influence of glutathione:

FIGURE 10: 7 **Metabolism of Ba 679 BR (10 µM, I) by human liver microsomes and liver cytosol, influence of glutathione.**

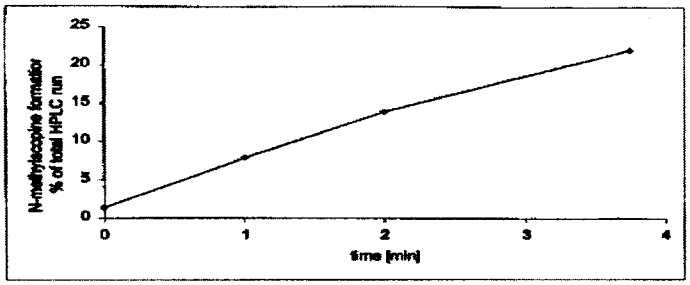


As shown in FIGURE 10: 7 Ba 679 BR was poorly metabolized by human liver cytosol and liver microsomes (during 60 min incubation). Glutathione had no effect on the velocity of Ba 679 BR metabolism.

Metabolism of Ba 679 by human liver microsomes - dependence of time:

FIGURE 10: 8 shows the increase of N-methylscopine over the time. N-methylscopine formation was in a linear range up to 2 h (per the sponsor, values were not corrected for the non-enzymatic hydrolysis of Ba 679 BR that was in the range of 4 - 5 %/h). Additional metabolites (retention times between 10 and 19 min) were found to an amount of<2% (data not shown).

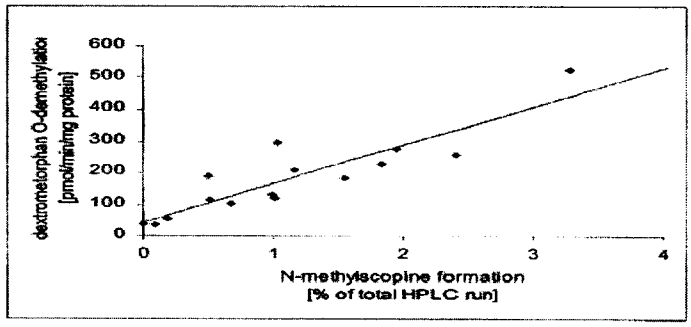
FIGURE 10: 8 **N-methylscopine formation by human liver microsomes. [^{14}C]Ba 679 BR (10 µM, I) was incubated with pooled human liver microsomes (0.5 mg protein/ml) in 0.1 M TRIS buffer in the presence of NADPH at 37°C for about 4 hours.**



Metabolism of Ba 679 BR by human liver microsomes - correlation with CYP 450 isoenzyme activity:

As shown in FIGURE 10: 9, increase of N-rnethylscopine correlaied with dextromethorphan O-demethylation (CYP 2D6, $r^2 = 0.794$).

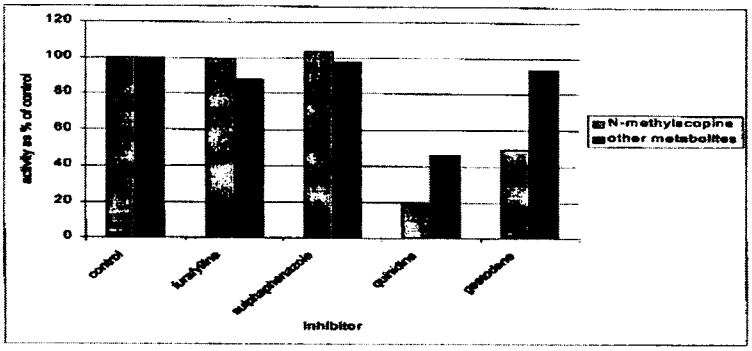
FIGURE 10: 9 **Ba 679 BR metabolism by human liver microsomes: correlation of N-methylscopine formation with dextromethorphan O-demethylation (CYP 2D6).**
[^{14}C]Ba 679 BR (100 µM, I) was incubated with human liver microsomes of fifteen individual donors.



Inhibition of Ba 679 BR metabolism by human liver microsomes by isoform selective CYP 450 chemical inhibitors:

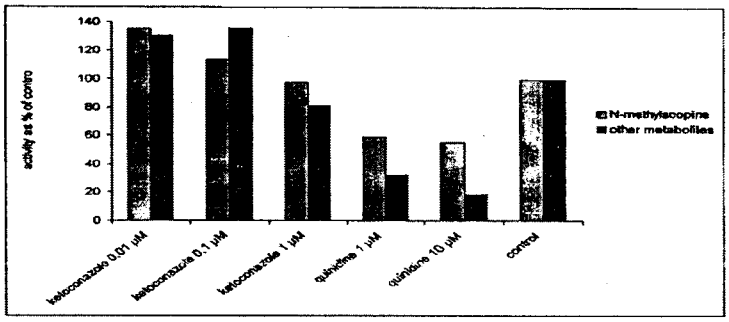
Quinidine inhibited the formation of N-methylscopine as well as the "other metabolites". Gestodene weakly inhibited the formation of other metabolites and N-methylscopine. Other chemicals did not influence Ba 679 BR metabolism (FIGURE 10:10).

FIGURE 10: 10 **Inhibition of Ba 679 BR (I) metabolism by human liver microsomes by CYP 450 isoform selective chemical inhibitors.**
Ba 679 BR (10 µM, I) was incubated with human liver microsomes (1 mg protein/ml) in the presence or absence of chemical CYP inhibitors for 90 min at 37°C.



Presence of ketoconazole (0.01, 0.1, 1.0 µM) resulted in a week inhibition of all metabolites. Quinidine (1, 10 µM) inhibited the formation of all metabolites (FIGURE 10:11).

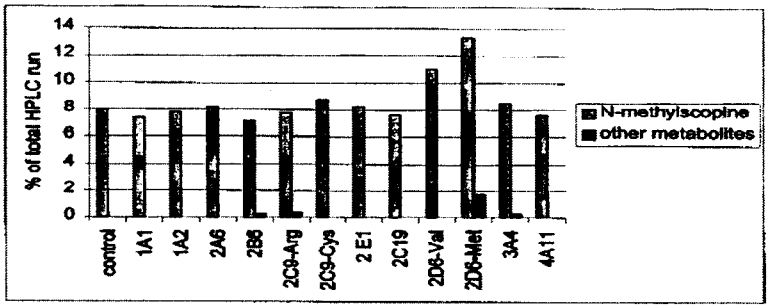
FIGURE 10: 11 **Inhibition of Ba 679 BR metabolism by human liver microsomes by ketoconazole (CYP 3A) and quinidine (CYP 2D6).**
Ba 679 BR (10 µM, I) was incubated with human liver microsomes (1 mg protein/ml) in the presence or absence of chemical CYP inhibitors for 90 min at 37°C.



Metabolism of Ba 679 BR by recombinant human liver CYP isoforms:

N-methylscopine formation, above the non-enzymatic background formation, was mediated only by CYP 2D6. Formation of other metabolites occurred in incubations with GYP 2D6 (2B6, 2C9, 3A4 <1 %) (FIGURE 10: 12).

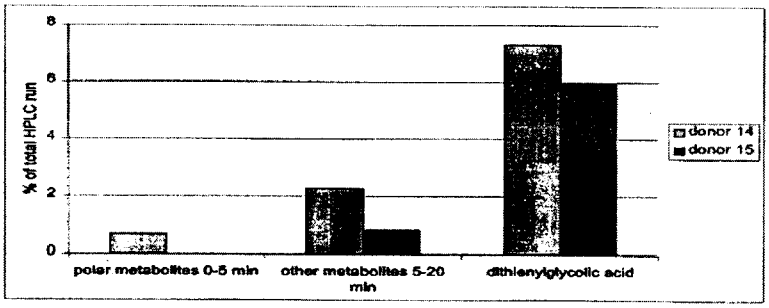
FIGURE 10: 12 **Metabolism of Ba 679 BR by human recombinant CYP isoforms.**
Ba 679 BR (1 µM, I) was incubated with human recombinant CYPs (1 mg protein/ml) in 0.1 M TRIS buffer pH 7.4 at 37°C for 2 h (mean of two experiments).



Metabolism of Ba 679 BR by human liver microsomes with high and low CYP 2D6 activities:

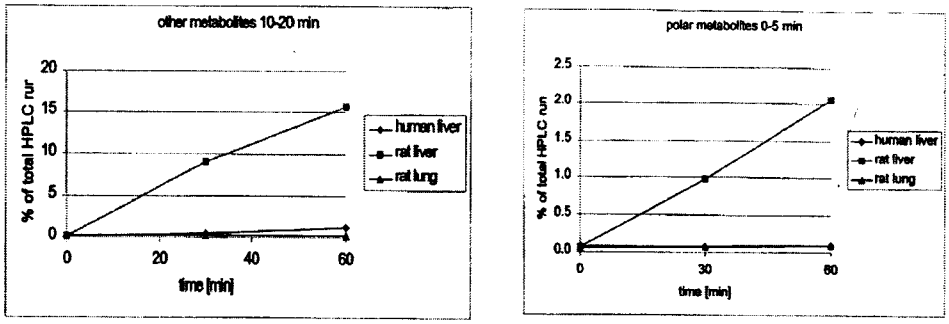
[^{14}C]Ba 679 BR (100 µM, II) was incubated with human liver microsomes of donor 14 and donor 15. Donor 14 had high, donor 15 low capacity for dextromethorphan O-demethylation CYP (CYP 2D6). As shown in FIGURE 10: 13, in incubations with microsomes of donor 15 polar metabolites (retention time = 0-5 min) were not formed and formation of other metabolites (retention time = 5-20 min) was lower than with microsomes of donor 14.

FIGURE 10: 13 **Metabolism of Ba 679 BR by human liver microsomes with high (donor 14) and low (donor 15) CYP enzyme activities.**
Ba 679 BR (100 µM, II) was incubated with human liver microsomes (1 mg protein/ml) of donor 14 and 15 in 0.1 M TRIS buffer pH 7.4 at 37°C for 90 min (mean of two experiments).



Metabolism of Ba 679 BR by rat lung microsomes: Very low metabolic activity was observed in rat lung microsomes and human liver microsomes compared to rat liver (FIGURE 10: 14).

FIGURE 10: 14 **Metabolism of Ba 679 BR by rat lung microsomes compared to human and rat liver microsomes.**
Ba 679 BR (100 µM, II) was incubated with human liver microsomes (0.5 mg protein/ml) in 0.1 M TRIS buffer pH 7.4 at 37°C for up to 60 min (mean of two experiments).



Binding of Ba 679 BR related radioactivity to microsomal protein:

During incubation experiments containing Ba 679 BR (100 µM. I and II, respectively) about 1 to 5 % of total radioactivity was bound to rat microsomal protein, dependent on the radioactive Ba 679 BR batch used (FIGURES 10:15 and 10:16). Binding to protein was dependent on NADPH and was higher with rat liver microsomes than with human liver microsomes (0.4 – 0.5%). Glutathione reduced binding to microsomal protein. KCN, used to trap metabolically generated electrophilic species, had only minor effects on binding to microsomal protein.

FIGURE 10: 15 **Binding of Ba 679 BR and its metabolites to microsomal protein. Ba 679 BR (100 µM, I) was incubated with human and rat liver microsomes (0.5 mg protein/ml) in 0.1 M TRIS buffer pH 7.4 at 37°C for 90 min (mean of three experiments).**

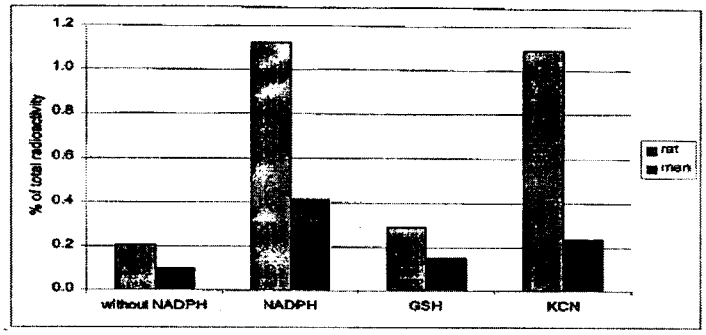
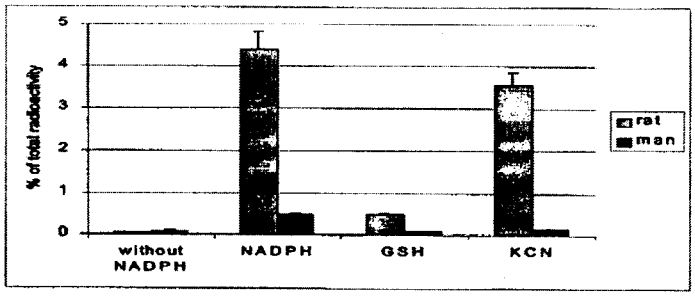


FIGURE 10: 16 Binding of Ba 679 BR and its metabolites to microsomal protein. Ba 679 BR (100 µM, II) was incubated with human and rat liver microsomes (0.5 mg protein/ml) in 0.1 M TRIS buffer pH 7.4 at 37°C for 90 min (mean of three experiments).



<u>Metabolism of dithienylglycolic acid by rat liver S9 supernatant of phenoharbital induced rats in the presence of gluthathione</u>:

FIGURE 10: 17 **Metabolism of dithienylglycolic acid by rat liver S9 supernatant of phenobarbital induced rats in the presence of glutathione. Dithienylglycolic acid (100 µM) was incubated with rat liver S9 supernatant in 0.1 M TRIS buffer pH 7.4 at 37°C for up to 30 min (mean of two experiments).**

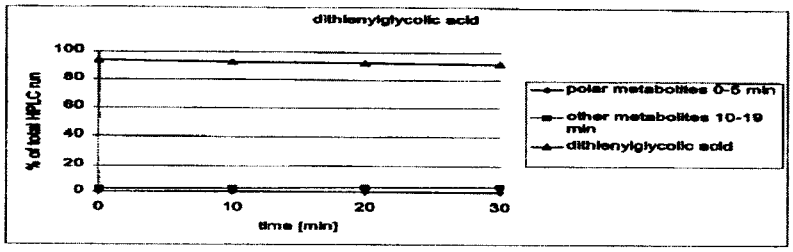
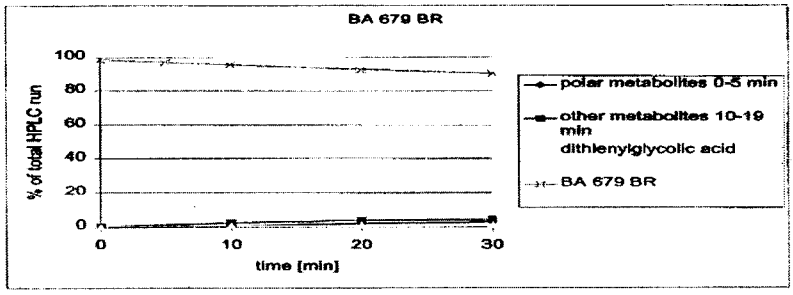


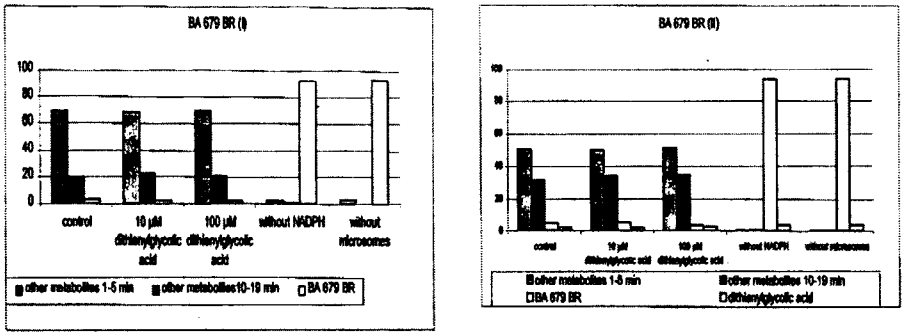
FIGURE 10: 18 **Metabolism of Ba 679 BR by rat liver S9 supernatant of phenobarbital induced rats in the presence of glutathione.**
Ba 679 BR (100 µM, II) was incubated with rat liver S9 supernatant in 0.1 M TRIS buffer pH 7.4 at 37°C for up to 30 min (mean of two experiments).



As shown in FIGURE 10: 17, dithienylglycolic acid was not metabolized, while Ba 679 BR decreased by about 10% (FIGURE 10: 18).

Influence of dithienylglycolic acid on Ba 679 BR metabolism by rat liver microsomes: Dithienylglycolic acid did not inhibit Ba 679 BR metabolism (FIGURE 10: 19)., and pre-incubation of dithienylglyeolie acid with microsomes in the presence of NADPH and further addition of Ba 679 BR had also no effect on Ba 679 BR metabolism (data not shown).

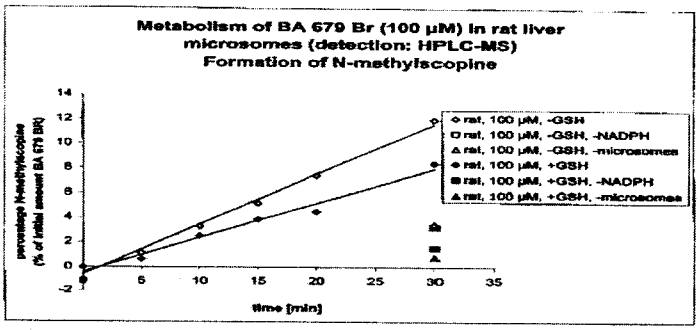
FIGURE 10: 19 **Influence of dithienylglycolic acid on Ba 679 BR (I: upper panel, II: lower panel) metabolism by rat liver microsomes.**
Ba 679 BR (10 µM, I and II) was incubated with rat liver microsomes (1 mg protein/ml) in the presence or absence of dithienylglycolic acid for 35 min at 37°C.



Influence of quercitrin on Ba 679 BR metabolism by rat liver tnicrosomes: [^{14}C]Ba 679 BR (100 µM, I) was incubated with rat liver microsomes (1 mg protein/mL) in 0.1 M TRIS buffer pH 7.4 in the presence of NADPH and in the presence of quercitrin (10 and 100 µM) at 37^{0}C for 90 mm. Quercitrin was used as an inhibitor of reductases in order to inhibit the reduction of a potentially formed keto-metabolite of N-methylscopine. Formation of N-methylscopine was 12.7 %, unchanged in the presence or absence of quercitrin (data not provided by the sponsor).

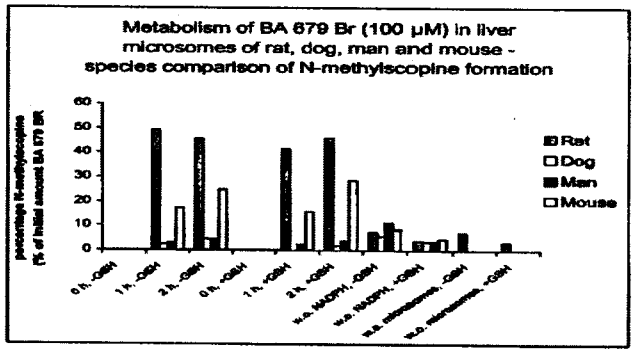
Metabolism of Ba 679 BR in rat liver microsomes – time-dependent formation of N-methylscopine in liver microsomes determined by HPLC-MS: N-methylscopine was detected in control incubations without NADPH or without microsomes (FIGURE 10: 26).

FIGURE 10: 26 Metabolism of Ba 679 BR (100 µM, I) by rat liver microsomes. Formation of N-methylscopine measured by HPLC. Solid lines represent the linear regression for the time dependent formation of N-methylscopine.



The amount of N-methylscopine formed in the incubation was species dependent (FIGURE 10: 27). N-methylscopine was not formed in the absence of microsomes in human liver, while N-methylscopine was formed without NADPH or microsomes in rats.

FIGURE 10: 27 Metabolism of Ba 679 BR (100 µM, I) by rat, dog, human and mouse liver microsomes. Formation of N-methylscopine measured by HPLC-MS.



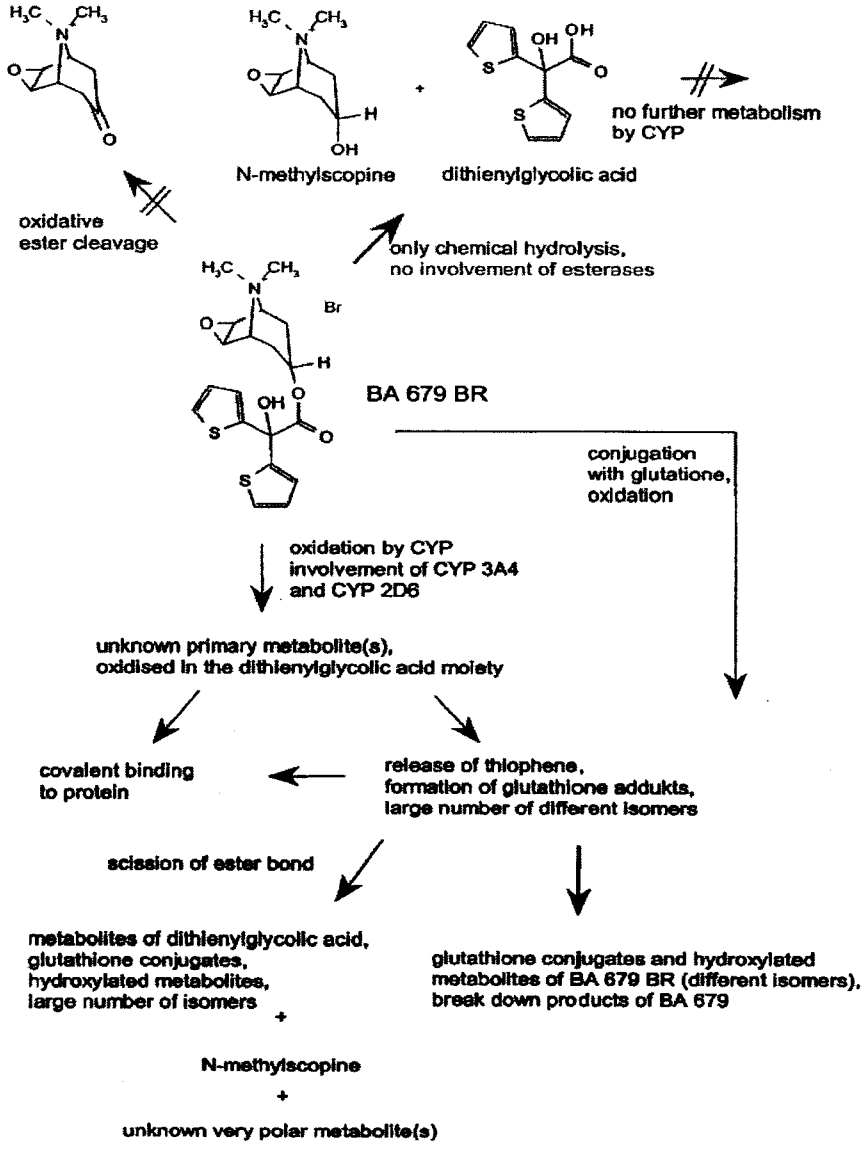
Summary:

- Metabolism is marginal in rat lung microsomes compared to rat liver microsomes.
- Ba 679 BR is metabolized by CYP leading to the metabolites observed in vitro, namely oxidation in the thiophene ring systems, glutathione conjugation and oxidative cleavage of thiophene ring systems. The site of metabolic attack was the dithienylglycolie acid moiety. Enzymatic cleavage of the ester linkage either by esterases or by CYP does not occur.
- Use of enzyme specific chemical inhibitors, recombinant CYPs, and correlation analysis showed the involvement of CYP 3A4 and CYP 2D6 in the metabolism of Ba 679 BR.
- Binding of radioactivity to microsomal protein in rat and human liver microsomes was dependent on NADPH indicating the formation of electrophilic metabolite(s). These metabolites could be trapped with glutathione resulting in lower binding of radioactivity to

microsomal protein in the presence of glutathione. Binding of Ba 679 BR metabolites was higher in rat liver microsoines than in human liver microsomes (rat: 1-5 compared to human: 0.4-0.5 %) (FIGURE 10: 15, 16). Note: It was concluded that recommendation (to the sponsor) of any futher study regarding the 'binding to microsomal protein' was not necessary.

- Metabolism products were N-methylscopine and two other metabolite fractions, a complex mixture of metabolites with medium polarity (designated "other metabolites) and a fraction of polar metaholite(s) that eluted at early retention times.

The following scheme summarises metabolic degradation of Ba 679 BR:

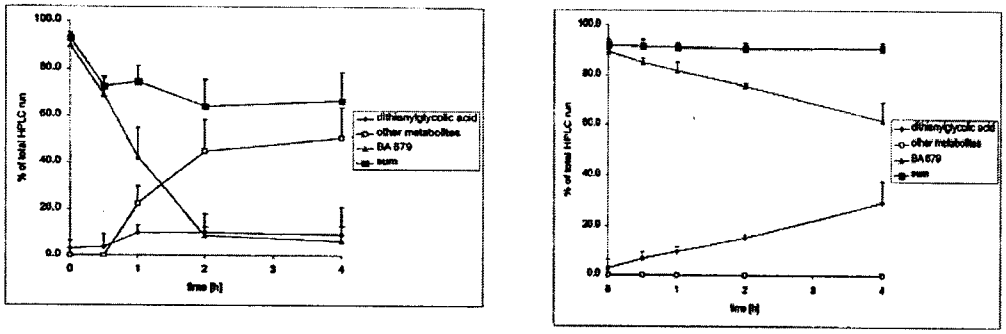


In vitro metabolism in human and animal hepatocytes (#99-1349)

Ba 679 BR bearing the [^{14}C]-label in the dithienylglycolic acid moiety (because the main site of metabolic attack are the thiophene residue of Ba 679 BR, per the sponsor) was incubated with human or rat hepatocytes.

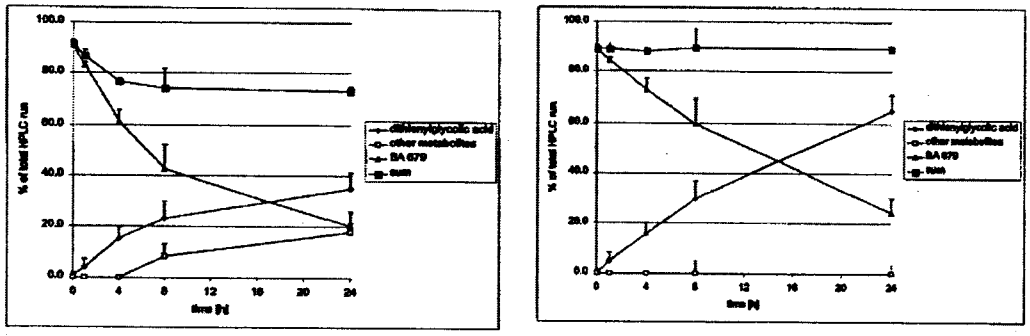
Incubation of Ba 679 BR with rat hepatocytes resulted in decrease of parent compound (after 2 hrs of incubation 1 and 10 µM Ba 679 BR decreased to 10 and 20%, respectively), formation of dithienylglycolic acid and the formation of other metabolites that were not separated by HPLC and therefore were quantitatively assessed as a single fraction, designated "other metabolites" (Figure 1). In control incubations without cells, only hydrolytic cleavage of the parent compound to dithienylglycolic acid took place (Figure 1).

Figure 1. Time dependence of Ba 679 BR (1 µM) metabolism by hepatocytes of male rats (left panel) and control incubation with collagen matrix without cells (right panel).



In human hepatocytes, HPLC patterns were similar to those of rat hepatocytes, but metabolism was slower (so it may provide a bigger amount of Ba 679 BR available for hydrolysis) (Figure 2).

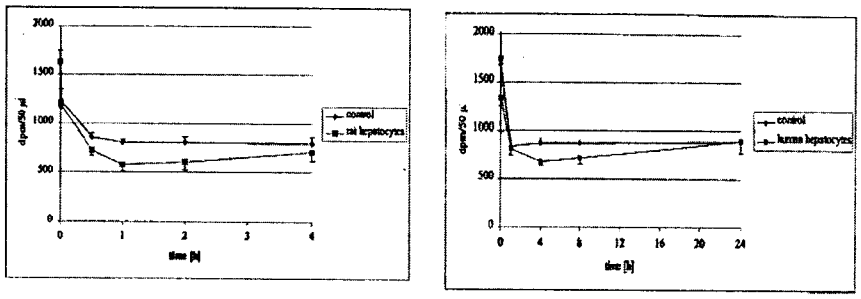
Figure 2. Time dependence of Ba 679 BR (1 µM) metabolism by human hepatocytes (left panel) and control incubation with collagen matrix without cells (right panel).



Both, in incubations with human and rat hepatocytes, a decrease of [^{14}C]radioactivity to about half of the initial value occurred within the first hour of incubation time (Figure 3). This may be due to the rapid distribution of Ba 679 BR into the cells and collagen matrix. In incubations containing 1 µM Ba 679 BR, radioactivity raised from 2 to 4 h and 4 to 24 h, for rat and human

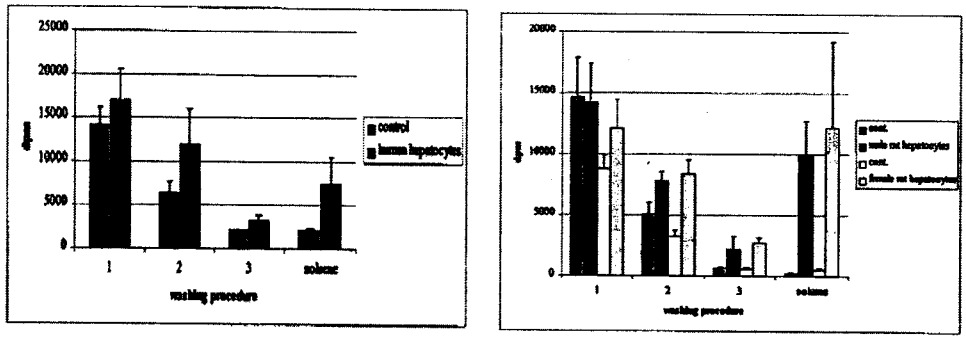
hepatocytes, respectively (Figure 3). This probably indicated the efflux of radioactive compounds out of the cells.

Figure 3. Time dependence of radioactivity in medium of hepatocyte culture of female rats (left panel) and human (right panel)



The results of radioactivity in cell pellets showed that 1.6 to 2.6 % of total radioactivity were bound to cell proteins of rat hepatocytes compared to about 0.8 to 0.9 % of human hepatocytes (Figure 4).

Figure 4. [^{14}C]radioactivity in the cell pellets of human (left panel) and rat hepatocytes (Ba 679 10 µM)



Conclusion:

- Ba 679 BR was metabolized by rat and human hepatocytes with the formation of dithienylglycolic acid and a variety of other metabolites.
- Formation of dithienylglycolic acid by non-enzymatic ester cleavage was predominant in human hepatocytes.
- Decrease of parent compound and formation of other metabolites was faster by rat hepatocytes (after 2 hours of incubation 1 and 10 µM Ba 679 BR decreased to 10 and 20%, respectively) compared to human hepatocytes (after 24 hours of incubation 1 and 10 µM Ba 679 BR decreased to 20 and 25%, respectively).
- About 2 % of total radioactivity were bound to rat hepatocytes compared to about 1 % in human hepatocytes.
- Identification of other metabolites by HPLC-MS was not done.

Comment: (long term) Effects of binding (covalent) to hepatocytes (and presumably to other cells) are worth to investigate for the safety.

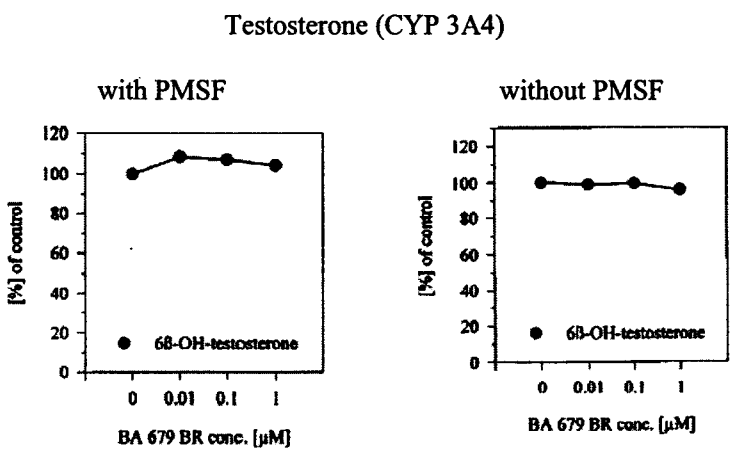
***In vitro* inhibition studies on cytochrome P450 dependent metabolic reactions (U97-2651)**

Inhibition of cytochrome P450 catalyzed test reactions by Ba 679 BR was investigated in liver microsomes of humans. The following test reactions were used. These test substrates are generally accepted as selective markers of the enzymatic activity of a single or two closely related cytochrome P450 isoenzymes that are relevant for drug metabolism in humans: phenacetin O-dcatkylation (test for cytochrome P450 1AI and 1A2); S-mephenytoin N-dealkylation (test for cytochrome P450 2B6); S-mephenytoin 4'-hydroxylation (test for cytochrome P450 2C19); tolbutamide hydroxylation (test for eytochrome P450 2C9); bufuralol 1'-hydroxylation (test for cytochromc P450 2D6); chlorzoxazone 6-hvdroxylation (test for cytochrome P450 2E1); nifedipine oxidation (test for cylochrome P450 3A); testosterone 6β-hydroxylation (test for cytochrome P450 3A)

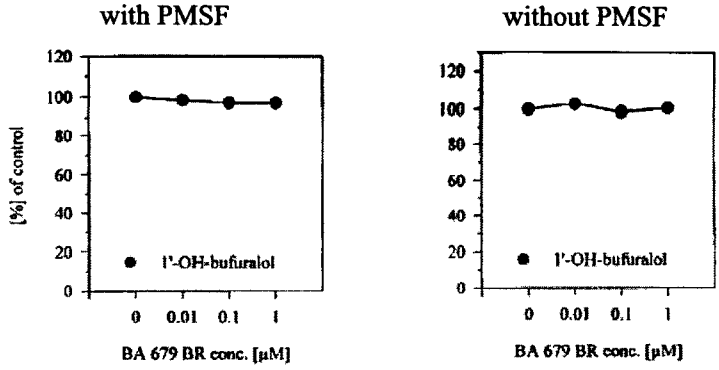
The test substrates were incubated with human liver microsomes in the presence NADPH and the formation of the respective metabolites was quantified. Ba 679 BR was added to the incubation experiments and its effect on the formation of the respective metabolites was evaluated. Quantification was done by using [^{14}C]-radiolabelled test substrates and formed metabolites were quantified by [redacted] detection. The extent of inhibition by Ba 679 BR was assessed at 3 different Ba 679 BR concentrations (0.01, 0.1 and 1 µM). In addition, in order to avoid esterase-catalyzed degradation of Ba 679 BR during the incubation experiment, several test reactions were performed in the presence and absence of the potent esterase inhibitor PMSF.

Results: No relevant effects on cytochrome P450 catalyzed test reactions were observed at any concentration of BA 679 BR. There was also no clear and consistent difference between test reactions that were performed with or without PMSF. Figure 1 shows the results graphically (limited to testosterone and bufuralol).

Figure 1. Effect of Ba 679 BR on different P450 test reactions. Experiments were performed with pooled human liver microsomes and 3 different concentrations of Ba 679 BR with or without PMSF. Data on product formation relative to control incubation (%) are shown.



Bufuralol CYP 2D6



Conclusion:

Ba 679 BR exhibited no inhibition of several CYP 450 catalyzed test reactions. Therefore, the sponsor concluded that clinically relevant drug-drug interactions, based on metabolic inhibition of other drugs that are substrates of cytochrome P450 enzymes by therapeutic doses of Ba 679 BR, are unlikely to occur. The sponsor made an adequate conclusion.

Stability of tiotropium bromide in plasma (#U91-0236)

Tiotropium bromide is stable in acidic aqueous solutions (pH 2). The hydrolytic cleavage becomes more rapid with increasing pH values and had a hydrolysis half-life of 17 h at 37 °C in pH 7.4 rat plasma as well as in 0.1 M phosphate buffer pH 7.25. The hydrolysis of the ester bond was temperature-sensitive as the rate of hydrolysis was threefold lower at 25°C.

Protein binding in human plasma (U99-1707)

The plasma protein bind was determined by ultrafiltration of the human plasma using [^{3}H]tiotropium concentrations of 10-300 pg/mL. The mean extent of binding of [^{3}H]tiotropium to human plasma was 73, 71 and 72% at drug concentrations of 10, 50 and 300 pg/mL, respectively (the mean overall binding to human plasma protein was 72 ± 1.1%). Thus, the extent of binding of [^{3}H]tiotropium was independent of the drug concentration used.

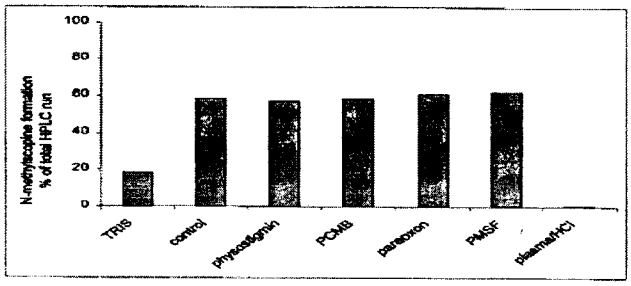
Comaprison of tiotropium bromide hydrolysis by plasma samples of various animal and species and human plasma (U98-2865)

BA 679 BR is an ester composed of N-methylseopine and dithienylglycolic acid. Apart from chemical hydrolysis the ester linkage could also be susceptible to enzymatic cleavage by plasma esterases. This study was performed in order to investigate the contribution of plasma esterases in hydrolysis of BA 679 BR. In vitro investigations were performed with [^{14}C]BA 679 BR labelled in the N-methylscopine part using human and animal plasma (rat, dog, rabbit, and mouse). Increase of N-methylscopine and decrease of parent compound, BA 679 BR were measured during incubation experiments with BA 679 BR in plasma samples of the above

mentioned species. Time-dependent cleavage of BA 679 BR was observed in protein-free buffer as well as in native plasma samples of the above mentioned animal species.

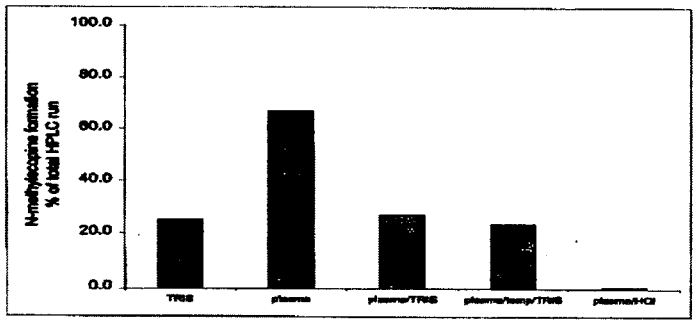
Inhibition of enzymatic ester hydrolysis in plasma samples: Various esterase inhibitors, physostigmine, paraoxon, PMSF, PCMB, BEA 2108 BR (structurally related compound to BA 679 BR with the N-methylscopine part replaced by N-methyltropan), BNPP, and iso-OMPA were pre incubated before addition of substrate. Aliquots were taken and the reaction was terminated by addition of an equal volume of 0.2 M HCL and cooling ice. As shown in Figure 3, none of the inhibitors was able to prevent hydrolysis of tiotropium.

Figure 3: Influence of potential esterase inhibitors on BA 679 BR in human plasma. Incubation experiments were performed at 37°C for 4 h (pre-incubation: 5 min at 37°C, mean of two experiments).



Incubation of BA 679 BR (tiotropium) with heat activated human plasma: BA 679 BR (10 µM) was incubated with heat activated human plasma (5min/80°C), containing 0.1 M TRIS buffer, with human plasma, and with human plasma containing 0.1 M TRIS buffer at 37°C for 4 h. Figure 6 shows the different rates of hydrolysis.

Figure 6: Incubation of BA 679 BR with heat inactivated human plasma (5 min 80°C and plasma, diluted with TRIS buffer (4 h at 37°C).



Conclusions: Hydrolysis of BA 679 BR was observed in plasma samples, however, esterases present in plasma have no effect on hydrolysis of BA 679 BR.

Office of Clinical Pharmacology and Biopharmaceutics

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA Number	21-395	Brand Name	Spiriva®
OCPB Division (I, II, III)	DPE-II	Generic Name	Tiotropium bromide
Medical Division	HFD-570	Drug Class	Anticholinergic
OCPB Reviewer	Shinja Kim	Indication(s)	COPD
OCPB Team Leader	Emmanuel Fadiran	Dosage Form	Inhalation Powder Capsule
		Dosing Regimen	1 capsule QD
Date of Submission	12/13/01	Route of Administration	Oral Inhalation
Estimated Due Date of OCPB Review	8/13/02	Sponsor	Boehringer Ingelheim
PDUFA Due Date	10/13/02	Priority Classification	S
Division Due Date	9/13/02		

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	x			
Tabular Listing of All Human Studies	x			
HPK Summary	x			
Labeling	x			
Reference Bioanalytical and Analytical Methods	x			
I. Clinical Pharmacology				
Mass balance:	x			Reports/in vitro studies regarding metabolism (animal) protein binding, etc..
Isozyme characterization:	x			
Blood/plasma ratio:	x			
Plasma protein binding:	x			
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	x	5		
multiple dose:	x	3		
Patients-				
single dose:	x	2		
multiple dose:	x	5		
Dose proportionality -				
fasting / non-fasting single dose:	x			
fasting / non-fasting multiple dose:	x			
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				
In-vitro:				
Subpopulation studies -				
ethnicity:	x			African-American *vs.* Caucasian
gender:	x			
pediatrics:				
geriatrics:	x			
renal impairment:	x			
hepatic impairment:		x		
PD:				
Phase 2:	x			
Phase 3:	x			
PK/PD:				
Phase 1 and/or 2, proof of concept:	x			Dose-response
Phase 3 clinical trial:	x			Dose-response

Population Analyses -				
Data rich:				
Data sparse:				
II. Biopharmaceutics				
Absolute bioavailability:	x			
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:				
replicate design; single / multi dose:				
Food-drug interaction studies:				
Dissolution:				
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies		15		Exclude in vitro studies

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/s/

Shinja Kim
9/18/02 09:42:16 AM
BIOPHARMACEUTICS

Emmanuel Fadiran
9/18/02 09:51:07 AM
BIOPHARMACEUTICS
I concur